IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.:	**09/ 436,347**	Group Art Unit:	1643
Confirmation No.:	6491	Examiner:	A.M. Harris
Filed:	9 November 1999		
Inventor:	Christine A. WHITE *et al.*		

For: Treatment of Chronic Lymphocytic Leukemia using Anti-CD20 Antibodies (as amended)

DECLARATION OF DAVID P. SCHENKEIN, M.D., UNDER 37 C.F.R. § 1.132

1. I am employed by Genentech, Inc., as Senior Vice President, Clinical Hematology/Oncology. Genentech, a licensee of the patent application identified above, co-promotes RITUXAN® (rituximab), a therapeutic CD20 antibody, in the United States together with Biogen Idec Inc., the owner of the patent application.

2. I am board certified in Internal Medicine and Hematology, specializing in the field of hematologic malignancies. Since 1986, I have been involved in clinical trials for treatments of hematologic malignancies, both at academic research hospitals and at biopharmaceutical companies. A copy of my curriculum vitae is attached.

3. In 1998, I was active in treating cancer patients, including patients having chronic lymphocytic leukemia (CLL), at New England Medical Center. I also held an appointment as Associate Professor of Medicine at Tufts University School of Medicine.

4. I have reviewed the patent application identified above, the claims now pending, the Office action dated May 29, 2008, and the references cited in the Office action, specifically:

   - RITUXAN® (rituximab) package insert dated November, 1997
   - McLaughlin *et al.*, *J. Clin. Oncol.* 16(8): 2825-33 (August, 1998)

- US Patent No. 5,736,137 ("the '137 patent")
- US Patent No. 5,843,398 ("the '398 patent")
- US Patent Application Publication No. 2003/0018014 ("the '014 application")
- Stenbygaard *et al.*, *Breast Cancer Research and Treatment* 25: 57-63 (1993)
- US Patent No. 6,090,365 ("the '365 patent")[1]

5. I understand that the Patent Office concludes that the claimed methods for treating CLL would have been obvious from the references listed in the paragraph above to a person of ordinary skill in the field of the invention in November, 1998.

The references cited in the Office action

Uses of anti-CD20 antibodies to treat low grade or follicular NHL

6. Rituximab (RITUXAN®), a chimeric (mouse-human) CD20 antibody, was approved by the Food and Drug Administration in November 1997. As the RITUXAN® package insert indicates, the original approval was limited to the treatment of patients with relapsed or refractory low-grade or follicular, CD20 positive, B-cell non-Hodgkin's lymphoma (NHL) (see "Indications and Usage").

7. McLaughlin *et al.* reports the results of the phase III clinical trial supporting the 1997 rituximab NHL approval. Rituximab was administered as a single agent to adult patients who had either not responded to, or relapsed from, primary therapy for low grade or follicular B-cell NHL. In this trial, the antibody dose was 375 mg/m^2, administered intravenously once weekly for a total of four infusions.

8. The clinical trial reported in the McLaughlin paper specifically *excluded* patients with CLL. CLL patients, defined as those having with lymphocyte counts > 5 x 10^9/L, were not eligible for the trial. *See* page 2826, column 2. This categorization employs a standard diagnostic criterion for CLL, as discussed below.

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[1] I am informed that the '398 patent and '365 patents, both naming Kaminski as a first inventor, share a common disclosure apart from the claims. For simplicity, I refer exclusively to the '365 patent in this declaration.

9. The '137 patent describes phase I/II single- and multiple-dose clinical trials with rituximab, termed C2B8 in the '137 patent, for patients having histologically confirmed B cell lymphoma. The '137 patent does not mention CLL.

The Kaminski patent

10. The therapeutic approach described in the '365 patent differs in significant respects from the one described in this application.

11. First, the '365 patent advocates radioimmunotherapy (*i.e.*, the use of an antibody to deliver a therapeutic radioisotope to target B-cells) as a therapeutic approach. In contrast, the approach described in this application uses an unmodified CD20 antibody, such as rituximab, to deplete B-cells. In fact, the '365 patent emphasizes the benefit of using a radiolabeled antibody as the therapeutic agent, referring to the "limited efficacy of unmodified antibodies" ('365, column 2, lines 22-24).

12. Second, in every passage where the '365 patent discusses the use of CD20 antibodies, CD20 radioimmunotherapy is described for treating B-cell *lymphoma*, rather than chronic lymphocytic *leukemia*. The title, abstract, field of the invention, summary of the invention, examples, and claims of the '365 patent all refer specifically to the use of CD20 antibodies to treat B-cell lymphomas, not CLL or any other leukemia.

13. The '365 patent includes a brief discussion that antibodies targeting B-cell antigens other than CD20 might be used to treat a variety of B-cell malignancies that are not lymphomas, including ALL, CLL, Hairy Cell leukemia, and chronic myeloblastic leukemia. *See* column 6, lines 10-18. With regard to these diseases, the patent mentions specifically the antibodies B2 (an antibody directed against CD21), B3 (CD22), B4 (CD19), and J5 (CD10/ CALLA). (The binding specificities of these antibodies at described at column 4, lines 42-64.) I read the passage at column 6 to convey the possibility that these antibodies *might* be tried for cancers other than lymphoma. The passage, however, does not provide sufficient guidance to inform an oncologist which antibody should be tested for which type of cancer.

14. A paragraph that appears in the '365 patent at column 8, lines 12-47, discusses the cellular and tissue distribution of the antigen recognized by the B1 antibody (*i.e.*, CD20). This paragraph does not discuss any therapeutic strategy related to the B1 antibody. I read this passage as simply characterizing the biology of CD20. While the passage notes that CD20 is expressed on CLL cells, it does not comment on the significance of that observation, and it does not discuss the relative expression levels of the antigen on any cell types. I do not believe that this passage would have suggested any therapeutic strategies to an oncologist in 1998.

Use of chemotherapy for cancer

15. The two remaining references, the '014 application and Stenbygaard, simply refer to the use of chemotherapy in cancer treatment. Neither reference mentions the use of a CD20 antibody. Stenbygaard does not even mention the management of CLL.

The combined teachings of the references

16. In my opinion, all of the information presented in the references cited in the Office action, taking into account the general knowledge and experience of practitioners in the field at the time, would not have given a person of ordinary skill in oncology in 1998 a reason to try to treat CLL with a CD20 antibody. Also, in my view, even if one or more of the references had proposed investigating such a therapeutic approach, the references would not have given a person of ordinary skill a reasonable basis to expect that such an approach would be effective.

17. An oncologist in 1998 would have approached the cited references with the view that chronic lymphocytic leukemia and non-Hodgkin's lymphoma are distinct diseases. Because of this, an oncologist reading the references would have drawn a clear distinction between treatments or proposed treatments of CLL patients, on the one hand, and treatments or proposed treatments of NHL patients on the other.

Differences between CLL and NHL

18. The biology, clinical characteristics, treatment options, and natural histories for hematologic malignancies are discussed in detail in standard reference works, such as *Hematology*, 2nd edition, R. Hoffman, ed. (Churchill Livingstone, 1995) ("Hoffman"), to which I refer below. Copies of the chapters I cite are provided as attachments to this declaration. As I explain below, the differences between CLL and NHL are manifested on several different levels.

– *Biology*

19. CLL and NHL are biologically distinct at the cellular level. Their unique cell biology is reflected in their characteristic phenotypic features. The main phenotypic features of B-cell CLL are a predominant population that share B-cell markers (CD19, CD20, and CD23) with the CD5 antigen (a pan-T-cell marker); B cells that are monoclonal with regard to expression of either κ or λ immunoglobulin chains; and surface immunoglobulin of low density. These characteristics distinguish CLL from B-cell lymphomas, including NHL. *See* B.D. Cheson *et al.* (1996) *Blood* 87: 4990-97, "National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment" (attached), at page 4990, column 2.

20. CLL cells express much lower levels of CD20 antigen than NHL cells. *See*, for example, Almasri *et al. Am. J. Hematol.* 40: 259-263 (1992) (attached). Almasri *et al.* note that reduced expression of CD20 antigen is a characteristic marker for CLL.

21. B-cell malignancies arise from the aberrant proliferation of pre-B- and B-cells at different stages of development. A given B-cell tumor thus retains certain biological characteristics of a particular stage of B-lymphoid cell differentiation. The antigen expression profiles of CLL and NHL cells are one indication that these tumor types originate from different cell types. In part because of their different origins, the cellular physiology of CLL tumor cells is distinct from the cellular physiology of NHL tumor cells. The differences in cellular physiology extend to differential sensitivity to candidate therapeutic agents.

– *Clinical presentation and diagnosis*

22. CLL is characterized by a much higher tumor burden (*i.e.*, significantly higher numbers of circulating tumor cells) than is NHL. The clinical diagnosis of CLL requires an absolute lymphocytosis with a lower threshold of $> 5 \times 10^9$ mature-appearing lymphocytes per liter of peripheral blood. This diagnostic criterion serves in part to distinguish CLL from small lymphocytic lymphoma (SLL). *See* Cheson *et al.*, page 4990, column 2.

23. CLL and NHL also typically affect different patient populations. CLL is predominantly a disease of older adults. The median age at diagnosis (as reported in 1995) was 55 years. *See* Hoffman, Chapter 83, page 1388, col. 1. NHL, on the other hand, is observed across a broader age range, although the incidence of NHL increases with age. *See* Hoffman, Chapter 81, page 1278, col. 2.

– *Treatment options*

24. Clinicians approach CLL and NHL with different expectations for therapy and different treatment plans. As the present patent application indicates, the standard of care in the late 1990s for the treatment of CLL was chlorambucil or fludarabine chemotherapy. These drugs can achieve high rates of partial remissions, but they are not curative. In contrast, the usual treatment of choice in the late 1990s for NHL was a chemotherapy regime, CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), used in combination with rituximab when that became available.

– *Natural history and outcome*

25. As noted in the paragraph above, in the late 1990s CLL was generally regarded as incurable, whereas NHL could be cured in a significant number of cases. The progression of each disease is unique as well.

26. CLL usually presents as an indolent disease, with patients often maintaining a generally stable clinical status for a relatively long period of time (*e.g.*, on the order of years). CLL patients are often asymptomatic or minimally symptomatic at diagnosis, and oncologists may choose not to initiate antineoplastic therapy until the

patient's symptoms progress. *See* Hoffman, Chapter 83, pages 1310-14. Although chlorambucil and fludarabine therapies often provide therapeutic benefit, their widespread use has not significantly affected the natural history of CLL in the patient population at large. As Hoffman noted in 1995, the overall median survival time for CLL patients had remained at about 6 years for decades. Chapter 83, page 1318, col. 2.

27. NHL, on the other hand, typically progresses much more rapidly than CLL. Patients with low grade/follicular NHL typically respond well to chemotherapy or radiotherapy, but patients with advanced disease frequently relapse after a period of time. The course of the disease is typically characterized by repeated cycles of treatment, remission, and relapse, with the time to relapse becoming shorter with each successive cycle. The progress and outcome of NHL depends strongly on the particular subtype of the lymphoma and the stage of the disease at which therapy is initiated. *See* Hoffman, chapter 81, pages 1284-86.

Treatment of CLL with a CD20 antibody

28. As I have noted, none of the references cited in the Office action suggests that CD20 antibodies will be useful to treat CLL. And, for the reasons I discuss in section above, oncologists would have regarded CLL and NHL as distinct with respect to their cell biology, clinical characteristics, and responsiveness to therapy. Because these disease types would not have been considered comparable, a person of ordinary skill would not have viewed prior clinical experience from the treatment of NHL using rituximab (as described in the RITUXAN® package insert, McLaughlin *et al.*, and the '137 patent) or radiolabeled B1 (as described in the '365 patent) as sufficient to establish a reasonable expectation that using the same antibodies to treat CLL would result in similar therapeutic benefit.

29. As I note above, low levels of CD20 expression are characteristic of CLL tumor cells. Based on the knowledge in the field regarding treatments of other tumor types, a person of ordinary skill would reasonably have expected that the efficacy of a target-specific drug would depend on the number of target molecules accessible on the surface of the tumor cell, since that would dictate the number of drug molecules that

could interact with each tumor cell. Although it was known at the time that most CLL cells express some CD20 antigen, it was not known whether the CD20 density on CLL cells would be sufficient to support efficient inhibition or killing of the CLL cells in a clinical context.

30. As shown for example by Cheson *et al.*, as discussed above, and in this application (*e.g.*, at paragraph 0080), CLL is also characterized by a high number of tumor cells in the blood, relative to other hematologic malignancies. In the absence of information such as that provided in the present patent application, this characteristic of CLL would have made treatment using CD20 antibodies less predictable, at least because the very high numbers of CD20+ cells would have created a huge "sink" of CD20 binding sites in the blood of CLL patients. The existence of such an antibody-binding sink would have led to uncertainty as to whether CD20 antibody therapy would be effective in such patients.

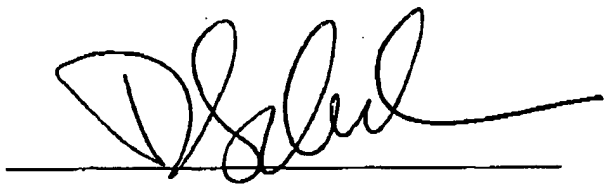
31. The combination of low CD20 density and high numbers of circulating tumor cells that is characteristic of CLL would have magnified the unpredictability due to either factor alone, from the perspective of a person of ordinary skill in 1998, regarding the therapeutic efficacy of using CD20 antibodies to treat CLL. The combination of these factors would have raised significant questions concerning the feasibility of providing CD20 antibodies at a sufficient per-cell density to effectively deplete B-cells in a CLL patient.

32. The expectation that CLL patients would respond very differently than would NHL patients to CD20 antibody therapy explains why CLL patients were categorically excluded from the clinical trial reported in McLaughlin *et al.* Because oncologists in 1998 recognized that CLL and NHL are distinct diseases, they would have expected that including CLL patients in the trial described in that publication would have compromised the value of the data for assessing the efficacy of rituximab in NHL patients.

33. For all of these reasons, I consider that an oncologist in 1998 would not have read any of the cited references, or any combination of those references, to suggest treating CLL with CD20 antibodies. The evidence in the cited references is consistent with my opinion that an oncologist of ordinary skill would not have held a reasonable expectation that clinical results in NHL patients could be extrapolated to predict responses to similar therapies in CLL patients.

* * *

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of any patent granted on this application.



David P. Schenkein, M.D.

Date: 11/14/08

EXHIBIT A

CURRICULUM VITAE **(11/03/07)**

David P. Schenkein, MD
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San Francisco, CA 94110
Cell (415) 254-6535

EDUCATION

1983	MD, State University of New York Upstate Medical School, Syracuse, New York
1979	BA, Wesleyan University, Middletown, Connecticut cum laude in Chemistry

INDUSTRY APPOINTMENTS

2007-	Senior Vice President, Clinical Hematology/Oncology Genentech, Inc, South San Francisco, CA
2006-2007	Vice President, Clinical Hematology/Oncology Genentech, Inc, South San Francisco, CA
2005-2006	Senior Vice President, Clinical Research Millennium Pharmaceuticals, Inc, Cambridge, MA
2002-2005	Vice President and Head of Clinical Oncology Millennium Pharmaceuticals, Inc, Cambridge, MA
2001-2002	Senior Director and Head of Clinical Oncology, Millennium Pharmaceuticals, Inc, Cambridge, MA

HOSPITAL APPOINTMENTS

2001-	Attending Physician, Hematology Oncology New England Medical Center, Boston, MA
1998-2001	Director, Cancer Center, New England Medical Center
1992-2001	Director, Lymphoma Service, New England Medical Center
1992-2001	Assistant Director of Bone Marrow Transplantation New England Medical Center
1997-1998	Deputy Director and Associate Director for Clinical Affairs and Network Development, New England Cancer Center,

New England Medical Center

1995-1997 Associate Chief, Division Hematology-Oncology
New England Medical Center

1993-1997 Director, Ambulatory Services, Hematology-Oncology
New England Medical Center

1992-1997 Director, Special Hematology Laboratory, New England Medical Center

UNIVERSITY APPOINTMENTS

1998- Associate Professor of Medicine, Tufts University School of Medicine, Boston Massachusetts

1997-2001 Assistant Dean for Oncologic Affairs
New England Medical Center

1989-1997 Assistant Professor of Medicine, Tufts University School of Medicine, Boston Massachusetts

TRAINING

1987-1989 Research Fellow: Laboratory of Dr. John Coffin, Tufts University School of Medicine, Boston, Massachusetts

1986-1987 Clinical Fellow: Hematology-Oncology, Tufts-New England Medical Center, Boston, Massachusetts

1984-1986 Resident: Internal Medicine, Tufts-New England Medical Center, Boston, Massachusetts

1983-1984 Intern: Internal Medicine, Tufts-New England Medical Center, Boston, Massachusetts

SERVICE/APPOINTMENTS TO ACADEMIC ENVIRONMENT

New England Medical Center:

1999-2001 Member, Lifespan RI Cancer Center Steering Committee

1999-2001 Member, NEMC Technology Assessment Committee

1998-2001 Chair, Cancer Care Committee

1998-2001 Member, Medical Board

1998-2001	Member, Credentials Committee
1997-2001	Network Task Force, Lifespan
1997-2001	Managed Care Committee
1993-2001	Quality Assurance Monitor-Division of Hematology/Oncology
1992-2001	Member Intern Selection Committee, New England Medical Center
1991-2001	ECOG(Eastern Cooperative Oncology Group) Bone Marrow Transplant Principal Investigator, New England Medical Center
1991-2001	Member, ECOG Bone Marrow Transplant and Lymphoma Steering Committees
1989-2001	Attending Physician teaching of residents/fellows: 12 hours/week
1983-2001	Member of Medical House Staff Curriculum Committee, New England Medical Center

Tufts University School of Medicine:

1996-1997	Senior Medicine Residents Teaching Award, New England Medical Center
1995-	Faculty Advisor, Tufts University School of Medicine
1995-	Lecturer: Pharmacology Course (2 lectures/year) Tufts University School of Medicine
1993-	Lecturer: Hematology Course (2-3 lectures/year) Tufts University School of Medicine
1989-1993	Small group instructor: Hematology Course (8 session/year) Tufts University School of Medicine

Community:

2004-	Board Member, CAVU
1997-	Medical Director, The Neely House
1997-	Adivsory Board, The Wellness Community
1996-	Advisor, Channel 7 WHDH-TV, Healthcast Team

1994- Director of the Neely House Planning Committee
Board Member, The Cam Neely Foundation

Industry:

1999-2001	Advisory Board, Supergen, Inc
1999-2001	Advisory Board, Coulter Pharmaceuticals Inc

CLINICAL TRIALS EXPERIENCE (academia only)

2000-	Study Chairman, A Phase III Trial of ^{90}Y-Anti-CD20 Antibody for Patients with Relapsed or Transformed Non-Hodgkin's Lymphoma
1999-	Study Chairman, A Phase III Trial of CMA 676-Antibody for Patients with Relapsed Acute Leukemia
1999-	Study Chairman, A Phase III Trial of ^{131}I-Anti-B1 Antibody for Patients with Relapsed or Transformed Non-Hodgkin's Lymphoma
1998-	Study Chairman, A Phase III Trial of High Dose Chemotherapy and Peripheral Blood Stem Cell Autologous Transplantation as Initial Therapy for Patients with Poor Prognosis Non-Hodgkin's Lymphoma
1997-	Study Chairman, A Phase III Randomized Trial of Cytoxan/GCSF vs GCSF Mobilization of PBSC for Patients with NHL and HD, New England Medical Center
1996-	Study Chairman, A Phase III Randomized Trial of High Dose Chemotherapy and Peripheral Blood Stem Cell Autologous Transplantation vs CHOP as Initial Therapy for Patients with Poor Prognosis Non-Hodgkin's Lymphoma, Eastern Cooperative Oncology Group.
1995-	Study Chairman, Treatment of Relapsed or Plateau Phase Multiple Myeloma with High-Dose Chemo/Radiotherapy, Peripheral Blood Stem Cell Rescue, and G-CSF, Myeloma Stem Cell Study Group
1995-	Study Co-Chairman, Treatment of HIV Associated Lymphoma with CHOP and B4 Blocked Ricin
1993-	Study Chairman, A Phase II/III Randomized Trial of Peripheral Blood Stem Cells vs Bone Marrow as Rescue for High Dose Chemotherapy in Patients with Hodgkin's Disease and Non-Hodgkin's Lymphoma.

1993-	Study Chairman, A Phase II Trial of High Dose Chemotherapy and Peripheral Blood Stem Cell Autologous Transplantation as Initial Therapy for Patients with Poor Prognosis Non-Hodgkin's Lymphoma, Eastern Cooperative Oncology Group.
1993-	Study Chairman, A Phase I/II Dose Escalation Trial of CNOP Chemotherapy and GM-CSF as Therapy for Non-Hodgkin's Lymphoma.
1992-	Study Co-Chairman, A Phase II Trial of High Dose Chemotherapy with G-CSF Primed Peripheral Stem Cell Reconstitution after Hormonal Recruitment in Women with Metastatic Breast Cancer.
1991-	Study Co-Chairman, Phase II Trial of Local Immunotherapy for the Treatment of AIDS Associated Kaposi Sarcoma, New England Medical Center.
1991-	Study Co-Chairman, Treatment of Relapsing or Refractory Multiple Myeloma with High-Dose Chemo/Radiotherapy, Peripheral Blood Stem Cell Rescue, and GM-CSF, Myeloma Stem Cell Study Group
1989-1991	Co-Investigator, Autologous Bone Marrow Rescue Using Peripheral Stem Cell Harvest in Patients with Relapsed or Refractory Multiple Myeloma, New England Medical Center
1989-1991	Co-Investigator, Phase II Trial of Autologous Bone Marrow Transplantation and Subsequent Immunotherapy with Interferon Alpha 2B in Hodgkin's Disease and Hon-Hodgkin's Lymphoma, New England Medical Center and University of Connecticut Health Center
1989-	Co-Investigator, Trial of Chemotherapy and Autologous Bone Marrow Transplantation for the Treatment of Acute Non-Lymphocytic Leukemia, New England Medical Center and Brigham and Women's Hospital

RESEARCH EXPERIENCE

1992-2001	Development of PCR Based Techniques to Detect and Quantitate Minimal Residual Malignant B cells in Patients with non-Hodgkin's Lymphoma, Department of Medicine, Division of Hematology-Oncology, New England Medical Center.
1987-1991	Investigation of the Early Events in Retroviral Replication, Dr. John Coffin, Department of Molecular Biology, Tufts University School of Medicine
1983-1984	Serum Amyloid A Protein: A Study of Serum Amyloid A Protein and Interleukin I in the Rabbit Model, Dr. Donald Bornstein, SUNY Upstate Medical Center
1977-1979	Beta-Lactamases: Research Leading to the Discovery of the First Specific, Non-Beta Lactam Inhibitor of Beta-Lactamases, Dr. Rex Pratt, Chemistry Department, Wesleyan University

GRANT SUPPORT

1. Charlton Award, Tufts University School of Medicine, 1989-1990, $7500

2. NIH Clinical Investigator Award, KO8 CA01554-05,

   2/1/90- 1/31/95, Direct Costs $85,600/year

3. Industry Funding: Clinical and Laboratory Trials:

   Amgen, Immunex, Immunogen, Wyeth-Ayerst, Coutler, IDEC

   1994- present Direct Cost $150,000/year

4. NCI U10 CA07190-36, Eastern Cooperative Oncology Group, 5% effort

CERTIFICATIONS/LICENSURES

1991	Board Certified: Medical Oncology. ABIM # 107921
1988	Board Certified: Hematology. ABIM # 107921
1986	Board Certified: Internal Medicine. ABIM # 107921
1985	Massachusetts License # 54139. Expiration Date 5/22/03

PROFESSIONAL MEMBERSHIPS

1994-	American Society of Clinical Oncology
1993-	American Federation for Clinical Research
1992-	American Society of Hematology
1986-	American College of Physicians
1986-	Massachussetts Medical Society

EDITORIAL BOARDS

1997-2001	The Journal of Oncology, Index and Reviews

PUBLICATIONS (Peer Reviewed Journals)

1. Schenkein, DP, and Pratt, RF. Phenylpropynal, a Specific, Irreversible, Non-Beta-Lactam Inhibitor of Beta-Lactamases, *Journal of Biological Chemistry*, 255(1) 45-48, 1980.

2. Lastavica, CC, Snydman, DR, Schenkein, DP, Beradi, VP, Pariser, KM. Demonstration of Borrelia Burgdorferi in a patient with Chronic Lyme Arthritis: *Zentralbl Bakteriol Mikrobiol Hyg* 263(1-2): 288, Dec 1986.

3. Snydman, DR, Schenkein, DP, Beradi, VP, Lastavica, CC, Pariser, KM. Borrelia Burgdorferi in Joint Fluid in Chronic Lyme Arthritis: *Annals of Internal Medicine*, 104(6):798-800, June 1986.

4. Schenkein, DP, O'Neill, WC, Shapiro, J, Miller, KB. Accelerated Bone Formation Causing Profound Hypocalcemia in Acute Leukemia, *Annals of Internal Medicine*, 105: (3):375-378, Sept 1986.

5. Grace, ME, Schenkein, DP, and Pratt, RF. Kinetics and Mechanism of Inactivation of the RTEM-2 Beta-Lactamases by Phenylpropynal. Identification of the Characteristic Chromophore. *Journal of Biological Chemistry*, 262(35):16778-16785, 1987.

6. Hillyer, CD, Comenzo, RL, Miller, KB, Schenkein, DP, Tiegerman, KO. Prompt Engraftment Using Autologous Peripheral Blood Stem Cells For Double Autologous Bone Marrow Rescue. *American Journal of Hematology*, 36(2),152-153, Feb 1991.

7. Miller, KB, Schenkein, DP and Fogaren,T. An Assesment of the Safety and Tolerability of Immune Globulin Intravenous (human) Sandoglobulin Prepared in Sterile Water or 5% Dextrose at Various Concentrations. *Seminars in Hematology*, 29,(3 Suppl 2):109-11, July 1992.

8. Miller, KB, Schenkein, DP, Wasserman, RL. Safety and Tolerability of an Intravenous Immune Globulin at Various Concentrations in 5% Dextrose Injection or Sterile Water for Injection. *Clin Pharm,* 11(7):628-631. July 1992.

9. Schenkein, DP and Harris, N. Case Records of the Massachusetts General Hospital, *New England Journal of Medicine*, 326,255-264,1992.

10. Comenzo, RL, Malachowski, ME, Miller, KB, Erban, JJ, Schenkein, DP, Desforges,JF, and Berkman,EM. Engraftment with Peripheral Blood Stem Cells Collected by Large-Volume Leukapheresis for Patients with Lymphoma. *Transfusion*, 32, 729-731, Oct 1992.

11. Schenkein, DP. High-Dose Cancer Therapy: Pharmacology, Hematopoietins, stem cells. Edited by James O. Armitage and Karen H. Antman. *N Engl J Med* , 329(4):285-286, July 22, 1993.

12. Miller, KB and Schenkein, DP. Hematology, *JAMA*, 270,(2) 216-217, July 14, 1993.

13. Rowe, JM, Ciobanu, N, Ascensao, J, Stadtmauer, EA, Weiner, RS, Schenkein, DP, McGlave, P, and Lazarus, HM. Recommended Guidelines for the Management of Autologous and Allogeneic Bone Marrow Transplantation: A Report from the Eastern Cooperative Oncology Group,(ECOG) *Annals of Internal Medicine*, 120,(2)143-158, January 15, 1994.

14. Miller, KB, Schenkein, DP, Comenzo, R, Erban, JK, Fogaren, T, Hirsch, CA, Berkman, E, Rabson A. Adjusted-Dose Continuous-Infusion Cyclosporine A to Prevent Graft-Versus-Host Disease Following Allogeneic Bone Marrow Transplantation, *Ann Hematol*, 68,(1)15-20, Jan 1994.

15. Schenkein, DP, Dixon, P, Desforges, JF, Berkman, E, Erban, JK and Miller, KB. Phase I/II study of Cyclophosphamide, Carboplatin, and Etoposide and Autologous Hematopoietic Stem-Cell Transplantation with posttransplant interferon alfa-2b for Patients with Lymphoma and Hodgkin's Disease. *J Clin Oncol,* 12, 2423-2431, Nov 1994.

16. Mitus, AJ, Miller, KB, Schenkein, DP,Ryan, HF, Parsons, SK, Wheeler, C, Antin, JH, Improved Survival for Patients with Acute Myelogenous Leukemia, *J Clin Oncol*, 13,(3) 560-569, March 1995.

17. Comenzo, RL, Malachowski, ME, Miller,KB, Erban, JK, Schenkein, DP, Desforges, JF, and Berkman, EM. Large-Volume Leukapheresis for Collection of Mononuclear Cells for Hematopoietic Rescue in Hodgkin's Disease, *Transfusion*, 35,(1)42-45, Jan 1995.

18. Frenette, PS, Desforges, JF, Schenkein, DP, Rabson, A, Slapack, CA, and Miller, KB. Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) Priming in the Treatment of Elderly Patients with Acute Myelogenous Leukemia, *Am J Heme*, 49,(1)48-55,1995.

19. Schenkein, DP and Ahmed, E. Case Records of the Massachusetts General Hospital, A 65 Year Old Man with Mediastinal Hodgkin's Disease and a Pelvic Mass, *New Eng J Med*, 333, 784-791,1995.

20. McCann, JC ,Kanteti, R, Shilepsky, B, Miller, KB, Sweet, M, and Schenkein, DP. High Degree of Occult Tumor Contamination in Bone Marrow and Peripheral Blood Stem Cells in Patients Undergoing Autologous Transplantation for non-Hodgkin's Lymphoma. *Biology of Blood and Marrow Transplantation,* 2,(1) 37-43, Feb1996.

21. Przybylski, GK, Goldman, J, Ng,VL, McGrath, MS, Herndier, BG, Schenkein, DP, Monroe, JG, and Silberstein, LE. Evidence for Early B-Cell Activation Preceding the Development of Epstein-Barr Virus-Negative Acquired Immunodeficiency Syndrome-Related Lymphoma, *Blood*, 88,(12) 4620-4629, Dec 1996.

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Formerly PS-341) Therapy in Patients with Advanced Multiple Myeloma (MM): Characterization and Reversibility. Session Type: Oral Session. *ASH Meeting* 2003.

61. Paul G. Richardson, Bart Barlogie, James Berenson, Seema Singhal, Sundar Jagannath, David Irwin, S. Vincent Rajkumar, Teru Hideshima, Barbara Bryant, George Mulligan, Hugh Xiao, Dixie Esseltine, David P. Schenkein, Kenneth C. Anderson, SUMMIT/CREST Investigators. 1629] Prognostic Factors for Response Parameters and Overall Survival in Patients with Multiple Myeloma (MM) Following Treatment with Bortezomib. Session Type: Poster Session 741-I *ASH Meeting* 2003.

62. Sundar Jagannath, Brian G.M. Durie, Jeffrey Wolf, Arnold Berliner, Elber Camacho, Eli Gabayan, David Irwin, Jose Lutzky, Marti McKinley, Phyllis Potts, Susan Noble-Kempin, Beth Davis, Amitabha Mazumder, John Crowley, Joth Jacobson, David Schenkein. [1650] Bortezomib (VELCADE™, Formerly PS-341) as First-Line Therapy in Patients with Multiple Myeloma (MM). Session Type: Poster Session 762-I *ASH Meeting* 2003.

63. Jorge Cortes, Francis Giles, Susan O'Brien, Miloslav Beran, David McConkey, John Wright, David Schenkein, Gira Patel, Srdan Verstovsek, Odeal Pate, Moshe Talpaz, Hagop Kantarjian [4971] Phase II Study of Bortezomib (VELCADE™, Formerly PS-341) for Patients with Imatinib-Refractory Chronic Myeloid Leukemia (CML) in Chronic (CP) or Accelerated Phase (AP). Session Type: Publication Only. *ASH Meeting* 2003.

64. Sandra J. Strauss, Wai Lui, David Schenkein, Reshma Shringarpure, Kenneth Anderson, T. Andrew Lister, Simon P. Joel [3361] The Effect of Proteasome Inhibition with Bortezomib (Velcade™) in B-Cell Lymphoma Cell Lines. Session Type: Poster Session 581-III. *ASH Meeting* 2003.

65. Owen OConnor, John Wright, Craig H. Moskowitz, Jamie Muzzy, Barbara MacGregor-Cortelli, Paul Hamlin, David Straus, Elizabeth Trehu, David P. Schenkein, Andrew D. Zelenetz [2346] Promising Activity of the Proteasome Inhibitor Bortezomib (Velcade) in the Treatment of Indolent Non-Hodgkin's Lymphoma and Mantle Cell Lymphoma. Session Type: Poster Session 517-II . *ASH Meeting* 2003.

66. Maurizio Zangari, Bart Barlogie, Joth Jacobson, Erik Rasmussen, Michael Burns, Bob Kordsmeier, John D. Shaughnessy, Elias J. Anaissie, Raymond Thertulien, Athanasios Fassas, Choon-Kee Lee, David Schenkein, Jerome B. Zeldis, Guido Tricot. [830] VTD Regimen Comprising Velcade (V) + Thalidomide (T) and Added DEX (D) for Non-Responders to V + T Effects a 57% PR Rate among 56 Patients with Myeloma (M) Relapsing after Autologous Transplant. Session Type: Oral Session. *ASH Meeting* 2003.

67. Hank H. Yang, Regina Swift, Karen Sadler, Robert Vescio, Julian Adams, David Schenkein, James R. Berenson (Intr. by James Berenson)[826] A Phase I/II Trial of VELCADE™ and Melphalan Combination Therapy (Vc+M) for Patients with Relapsed or Refractory Multiple Myeloma (MM). Session Type: Oral Session. *ASH Meeting* 2003.

68. Sagar Lonial, Edmund K. Waller, Paul G. Richardson, Sundar Jagannath, Dixil Francis, Melissa Lehman, Claire Torre, Bart Barlogie, James R. Berenson, Seema Singhal, David P. Schenkein, Dixie-Lee W. Esseltine, Jessica Anderson, Leonard T. Heffner, Kenneth C. Anderson

[1632] Evaluation of the Degree of Thrombocytopenia and Associated Risk Factors Following Bortezomib Therapy for Relapsed Multiple Myeloma. Session Type: Poster Session 744-I. *ASH Meeting* 2003.

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70. J. S. Ross, D. Schenkein, I. Webb, G. Gray, J. Deeds, R. Meyer, A. McDonald, C. Sheehan, K. Gray. Expression of prostate specific membrane antigen in the neo-vasculature of non-prostate cancers. *Journal of Clinical Oncology*, 2004 *ASCO Annual Meeting Proceedings* (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 3110

71. S. Jagannath, B. Durie, J. Wolf, E. Camacho, D. Irwin, J. Lutzky, M. McKinley, E. Gabayan, J. Crowley, D. P. Schenkein; First-line therapy with bortezomib (formerly PS-341) in patients with multiple myeloma (MM). *Journal of Clinical Oncology*, 2004 *ASCO Annual Meeting Proceedings* (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 6551

72. J. D. Cavenagh, N. Curry, J. Stec, M. Drake, T. C. M. Morris, S. Agrawal, D. Esseltine, D. Schenkein, H. Oakervee. PAD therapy (bortezomib, doxorubicin and dexamethasone) for untreated multiple myeloma (MM). *Journal of Clinical Oncology*, 2004 *ASCO Annual Meeting Proceedings* (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 6550

73. A. M. Davies, P. N. Lara, D. H. Lau, P. C. Mack, P. H. Gumerlock, D. R. Gandara, D. Schenkein, J. H. Doroshow. The proteasome inhibitor, bortezomib, in combination with gemcitabine (Gem) and carboplatin (Carbo) in advanced non-small cell lung cancer (NSCLC): Final results of a phase I California Cancer Consortium study. *Journal of Clinical Oncology*, 2004 *ASCO Annual Meeting Proceedings* (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 7106

74. A. Goy, A. Younes, P. McLaughlin, B. Pro, J. Romaguera, F. Hagemeister, L. Fayad, E. G. Trehu, D. Schenkein, M. A. Rodriguez. Update on a phase (ph) 2 study of bortezomib in patients (pts) with relapsed or refractory indolent or aggressive non-Hodgkin's lymphomas (NHL). *Journal of Clinical Oncology*, 2004 *ASCO Annual Meeting Proceedings* (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 6581

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76. M. P. Fanucchi, R. J. Belt, F. V. Fossella, R. B. Natale, F. Robert, P. Fidias, K. Kelly, O. Kashala, D. P. Schenkein, J. H. Schiller. Phase (ph) 2 study of bortezomib ± docetaxel in

previously treated patients (pts) with advanced non-small cell lung cancer (NSCLC): Preliminary results. *Journal of Clinical Oncology*, 2004 *ASCO Annual Meeting Proceedings* (Post-Meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004: 7107

77. Owen O'Connor, John Wright, Craig Moskowitz, Barbara MacGregor-Cortelli, David Straus, Andrew Evans, Jane Winter, Omer Koc, Nancy Horvath, Susan Blumel, Julie Vose, David Schenkein, Andrew Zelenetz. 607- A Multicenter Experience with Single Agent Bortezomib in Non-Hodgkin's Lymphoma Reveals Marked Differences in Sub-Type Sensitivity to Proteasome Inhibition. Session Type: Oral Session. . *ASH Meeting* 2004.

78. Sandra J. Strauss, Lenushka Maharaj, Jim Stec, Anthony Boral, Elizabeth Trehu, David Schenkein, Simon P. Joel, T. Andrew Lister. [1386] Phase II Clinical Study of Bortezomib (VELCADE®) in Patients (pts) with Relapsed / Refractory Non-Hodgkin's Lymphoma (NHL) and Hodgkin's Disease (HD). Session Type: Poster Session 540-I. *ASH Meeting* 2004.

79. Eyal C. Attar, Daniel J. DeAngelo, Karen K. Ballen, Emily Learner, Elizabeth G. Trehu, David P. Schenkein, James D. Levine, Richard M. Stone, Philip C. Amrein. [1799] Phase I Dose Escalating Trial of Bortezomib (Velcade®) in Combination with Idarubicin and Cytarabine in Patients with Acute Myeloid Leukemia. Session Type: Poster Session 12-II. *ASH Meeting* 2004.

80.. M.V. Mateos, Joan Blade, J. Diaz Mediavilla, J.J. Lahuerta, M.J. Terol, J. Hernández, M.J. Moro, J. Bargay, J.M. Ribera, J. de la Rubia, A. Sureda, D. Carrera, F. de Arriba, L. Palomera, M. Hernández, J. García Laraña, A. Alegre, F. Prosper, P. Rivas, D.L. Esseltine, D. Schenkein, J.F. San Miguel. [3462] A Phase I/II National, Multi-Center, Open-Label Study of Bortezomib Plus Melphalan and Prednisone (V-MP) in Elderly Untreated Multiple Myeloma Patients. Session Type: Poster Session 732-III. *ASH Meeting* 2004.

81. Teru Hideshima, Pierfrancesco Tassone, Dharminder Chauhan, Kenji Ishitsuka, Constantine Mitsiades, Noopur Raje, Shaji Kumar, Makoto Hamasaki, Hiromasa Hideshima, Nikhil C. Munshi, Paul G. Richardson, David Schenkein, Kenneth C. Anderson. Jerome Lipper [2351] Targeting IKK Inhibits Multiple Myeloma (MM) Cell Growth in the Bone Marrow Microenvironment. Session Type: Poster Session 564-II. *ASH Meeting* 2004.

82. James D. Cavenagh, Rakesh Popat, Nikki Curry, Jim Stec, Treen C. Morris, Mary Drake, Samir Agrawal, Patricia Smith, David P. Schenkein, Dixie-Lee Esseltine, Heather Oakervee. [1478] PAD Combination Therapy (PS-341/Bortezomib, Adriamycin and Dexamethasone) for Previously Untreated Patients with Multiple Myeloma. Session Type: Poster Session 632-I. *ASH Meeting* 2004.

83. James Berenson, H. Yang, R. Swift, K. Sadler, R. Vescio, J. Adams, D. Schenkein. [209] Bortezomib in Combination with Melphalan in the Treatment of Relapsed or Refractory Multiple Myeloma: A Phase I/II Study. Session Type: Oral Session. *ASH Meeting* 2004.

84. Paul Richardson, P. Sonneveld, M. Schuster, D. Irwin, E. Stadtmauer, T. Facon, J. Harousseau, D. Ben-Yehuda, S. Lonial, H. Goldschmidt, D. Reece, J. San Miguel, J. Blade, M. Boccadoro, J. Cavenagh, W. Dalton, A. Boral, D. Schenkein, K. Anderson. [336.5] Bortezomib Demonstrates Superior Efficacy to High-Dose Dexamethasone in Relapsed Multiple Myeloma: Final Report of the APEX Study. Session Type: Oral Session. *ASH Meeting* 2004.

85. Sundar Jagannath, Durie Brian, Jeffrey L. Wolf, Elber Camacho, David Irwin, Jose Lutzky, Marti McKinley, Eli Gabayan, Amitabha Mazumder, John Crowley, David Schenkein. [333] A Phase 2 Study of Bortezomib as First-Line Therapy in Patients with Multiple Myeloma. Session Type: Oral Session. *ASH Meeting* 2004.

86. Stefan Faderl, Kanti R. Rai, John Gribben, Ian Flinn, John C. Byrd, David McConkey, David Schenkein, Dixie Esseltine, Mary L. Browning, Michael J. Keating.[4841] Phase 2 Study of Three Doses of Single Agent Bortezomib in Patients with Fludarabine-Refractory B-Cell CLL. Session Type: Publication Only. *ASH Meeting* 2004.

SELECTED RECENT INVITED LECTURES (academia only)

June 1995, Visiting Professor, Grand Rounds, Tel Aviv University, Israel

September 1995: Oncology Grand Rounds, University of Pennsylvania, "Role of high dose chemotherapy and detection of minimal residual disease in Non Hodgkin's Lymphoma."

November 1995: Grand Medical Rounds, Youville Hospital, "Matched unrelated donor transplant, myth or magic."

April 1996: Grand Medical Rounds, Interfaith Medical Center, Salt Lake City, UT, "Role of high dose chemotherapy in Non Hodgkin's Lymphoma."

May 21, 1996: American Society of Clinical Oncology, Invited discussant, Philadelphia, PA, "Impact of detection of minimal residual disease in breast cancer, neuroblastoma, and lymphoma."

May 24, 1996: Grand Medical Rounds, Bay State Medical Center, "Update on Non Hodgkin's Lymphoma."

June 1996: Grand Rounds, Hospital Cantonal Universitaire, Geneve, Switzerland, " Clinical and Molecular Aspects of High Dose Therapy in Non-Hodgkin's Lymphoma"

June 1996: Oncology Grand Rounds, Rush Cancer Institute, "Impact of minimal residual disease on therapy for Non Hodgkin's Lymphoma."

July 8, 1996: Grand Medical Rounds, Catabaras Medical Center, Concord, NC., "Role of high dose chemotherapy in Non Hodgkin's Lymphoma."

January 1997: Medical Grand Rounds, Carolina Medical Center, "An update on Non Hodgkin's Lymphoma"

September 1996: Medical Grand Rounds, Newton Wellesley Hospital, "Matched unrelated donor transplant, myth or magic."

November 1996: Grand Medical Rounds, Saints Memorial Medical Center, "Update on Non Hodgkin's Lymphoma."

Chairperson, Acute Leukemia Simultaneous Session, ASH, 1996.

April 1997: Medical Grand Rounds, Carolina Medical Center, "Matched Unrelated Marrow Transplantation"

May 1997, Medical Grand Rounds, New England Medical Center, "NHL, Lessons from the bench and the bedside"

June 1997, Invited speaker, Frontiers of Hematology, Toronto, Ontario, "The role of initial transplantation in high risk NHL".

July, 1997, Medical Grand Rounds, Hamad Medical Center, Qatar, "NHL, Lessons from the bench and the bedside"

August 1997, Medical Grand Rounds and Visiting Professor, University Hospital, Sate University of New York, Syracuse, "NHL, Lessons from the bench and the bedside"

September 1997, Hematology-Oncology Grand Rounds, Dartmouth-Hitchcock Medical Center, "The role of initial transplantation in high risk NHL".

December 1997, Medical Grand Rounds, Faulkner Hospital, "Matched Unrelated Marrow Transplantation"

February 1998, Medical Grand Rounds, Carney Hospital, "NHL, Lessons from the bench and the bedside"

March 1998, Invited Speaker, American Society of Blood and Marrow Tranplantation Meeting, Miami,FL, "If, When, and What: The role of initial transplantation in high risk NHL".

April, 1998, Medical Grand Rounds, Youville Hospital, "NHL, Lessons from the bench and the bedside"

June 1998, Invited Speaker, Quebec Oncology Society, "The role of initial transplantation in high risk NHL".

July 1998, Invited Speaker, Ninth International Symposium on Blood and Marrow Transplantation, "Early or Late Autotransplant for High Risk NHL", Dallas, TX.

October 1998, Invited Speaker, IV International Conference on Cancer in the Elderly, "Age Adjusted Leukemia Therapy", Rome, Italy.

November 1998, Invited Speaker, Alberta Lymphoma Symposium, "The role of initial transplantation in high risk NHL", Calgary, Canada

November 1998, Invited Speaker, Transplant Grand Rounds, Beth-Israel Deaconness Medical Center, "The role of initial transplantation in high risk NHL", Boston, MA

November 1998, Invited Speaker, US Army American College of Physician Meeting, "NHL an Update", Washington, DC

December 1998, Oral Presentation, American Society of Hematology Meeting, Molecular Detection of Tumor Sequences in PBPC Mobilized with either STEMGEN (SCF) plus FILGRASTIM or FILGRASTIM alone, Miami, FL.

January 1999, Invited Speaker, Vancouver Lymphoma Symposium, "The role of initial transplantation in high risk NHL", Calgary, Canada

April 1999, Medical Grand Rounds, Saint Memorial Hospital, "Gene Therapy", Lowell, MA

September, 1999, Medical Grand Rounds, Saint Vincents Hospital, "Role of Monoclonal Antibody Therapy for NHL", MA

October, 1999, Invited Speaker, United Resource Network Conference, "Bone Marrow Transplantation, an Overview", Newport, RI

October, 1999, Invited Speaker, United Resource Network Tele-Conference, "Role of BMT in NHL".

December 1999, Chair, Leukemia Scientific Session, American Society of Hematology Meeting, New Orleans, LA

December 1999, Invited Speaker, Cigna Network Tele-Conference,."Role of BMT in NHL".

February 2000, Invited Speaker, Supergen Conference, "Low Grade NHL, New Agents and Modalities", Boston, MA.

February 2000, Visiting Professor, University of Wisconsin Cancer Center, "Role of BMT in NHL", Madison, Wisconsin.

February 2000, Invited Lecturer, European Bone Marrow Transplant Meeting: "Innovation in Neutrpenia Management: Development of a Novel Cytokine", Innsbruck, Austria.

December 2002, Invited Lecturer, Ross Lecture: "Oncology Drug Discovery in the 21st Century", SUNY, Syracuse, NY

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EXHIBIT B

National Cancer Institute–Sponsored Working Group Guidelines for Chronic Lymphocytic Leukemia: Revised Guidelines for Diagnosis and Treatment

By Bruce D. Cheson, John M. Bennett, Michael Grever, Neil Kay, Michael J. Keating, Susan O'Brien, and Kanti R. Rai

IN 1988, THE National Cancer Institute–sponsored Working Group (NCI-WG) on chronic lymphocytic leukemia (CLL) published guidelines for the design and conduct of clinical trials in CLL with two major objectives: first, to facilitate comparisons of results of clinical trials in CLL by providing standardized eligibility, response, and toxicity criteria; and, second, to encourage a framework on which to evaluate new scientific studies related to our increasing understanding of the biology and immunology of this disease.[1] These guidelines were rapidly adopted by the majority of the clinical trials community, and were also used by the Food and Drug Administration during its evaluation process for the approval of fludarabine. The differences between these guidelines and those subsequently published by the International Working Group on CLL (IWCLL), which were general-practice recommendations[2] are listed in Table 1. For diagnosis, the NCI-WG requires a lymphocyte count of 5×10^9/L, which is lower than the 10×10^9/L required by the IWCLL, unless the lymphocytes are B cells and the bone marrow is involved. To be considered a complete remission (CR), the NCI-WG criteria specify that less than 30% lymphocytes must be present in the bone marrow, with a recommendation that the clinical significance of lymphoid nodules be assessed prospectively (Table 1); the IWCLL allows focal infiltrates or nodules in the bone marrow aspirate and biopsy for CR. The IWCLL uses a shift in clinical stage as the sole index of partial remission (PR), whereas the NCI-WG provides more specific criteria and recommends validation of the relevance of stage shift. The major differences were the well-defined criteria in the NCI guidelines regarding when to initiate therapy, hematologic toxicity, and other important components for clinical trials design.

The purpose of this report is to present those revisions as considered necessary in view of advances in the past 8 years. Many of these revisions evolved as the guidelines were used in a systematic fashion in large clinical trials and, also, with the experience following the use of newer, more effective agents, such as fludarabine.[3-9] Although this report will focus on those changes recommended by the NCI-sponsored CLL Working Group, it will include sufficient details from the original guidelines so that the reader would find it a complete document by itself without having to refer to the older version.

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Submitted November 9, 1995; accepted February 7, 1996.

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The initial NCI-WG Guidelines were primarily designed as recommendations for the conduct of clinical trials. An important addition is that these revisions will distinguish practice guidelines from research issues in the diagnosis, decision to treat, and monitoring response in patients with CLL (Table 2).

It is increasingly clear that a more biologically relevant staging and response assessment of patients is needed if we are to continue to make progress in defining clinically disparate patient subsets and generate more innovative and effective treatment options.

1. Diagnosis of B-CLL

1.1. *Peripheral Blood*

The clinical diagnosis of CLL requires an absolute lymphocytosis with a lower threshold of greater than 5,000 mature-appearing lymphocytes/μL in the peripheral blood, in part to separate CLL from small lymphocytic non-Hodgkin's lymphoma. Morphologically, the lymphocytes must appear mature. Nevertheless, it is common to find admixtures of larger or atypical cells, cells that are cleaved, as well as those consistent with prolymphocytes; however, the percentage that should be used to distinguish CLL from prolymphocytic leukemia (PLL) is controversial. A value of up to 55% is still consistent with the diagnosis of CLL.[10] The presence of greater than 55% prolymphocytes and/or greater than 15,000/μL of prolymphocytes establishes a diagnosis of primary PLL or progression to prolymphocytoid leukemia. However, the markers on these cells should be different (eg, PLL cells are negative for CD5 in half of the cases). Prospective assessment of the significance of the proportion of peripheral blood prolymphocytes, their phenotypic characteristics, and the patterns of clonal evolution remain important research questions. The peripheral blood should also be carefully examined to rule out a leukemic phase of mantle-cell lymphoma, another CD5$^+$ lymphoid malignancy.[11,12]

In the original guidelines,[1] a duration of lymphocytosis of at least 4 weeks was required to substantiate the diagnosis. Since the clinical features, histology, and phenotypic characteristics are sufficient to permit an accurate diagnosis of CLL in the majority of patients, only in rare patients with questionable or indolent/smoldering CLL is a reassessment of the lymphocyte count needed after $\geq$4 weeks.[13,14] The routine availability of peripheral blood lymphocyte immunophenotyping has facilitated the diagnosis of CLL in patients with a monoclonal lymphocytosis.[12,15,16] Three main phenotypic features define B-CLL: the predominant population shares B-cell markers (CD19, CD20, and CD23) with the CD5 antigen, in the absence of other pan–T-cell markers; the B cell is monoclonal with regard to expression of either κ or λ; and surface immunoglobulin (sIg) is of low density. Not only are these characteristics generally adequate for a precise diagnosis, but, importantly, they distinguish CLL from uncommon disorders such as PLL, hairy-cell leukemia, mantle-cell lymphoma, and other lymphomas.[12,15]

These guidelines have been proposed for B-CLL. There-

Table 1. Comparison of NCI-Working Group and IWCLL Guidelines for CLL

Variable	NCI	IWCLL
Diagnosis		
Lymphocytes (× 10^9/L)	>5; ≥1 B-cell marker (CD19, CD20, CD23) + CD5	≥10 + B-phenotype or bone marrow involved <10 + both of above
Atypical cells (%) (eg, prolymphocytes)	<55	Not stated
Duration of lymphocytosis	None required	Not stated
Bone marrow lymphocytes (%)	≥30	>30
Staging	Modified Rai, correlate with Binet	IWCLL
Eligibility for trials	Active disease (details in document)	A: lymphs >50 × 10^9/L doubling time <12 mo diffuse marrow B, C: all patients
Response criteria		
CR		
Physical exam	Normal	Normal
Symptoms	None	None
Lymphocytes (× 10^9/L)	≤4	<4
Neutrophils (× 10^9/L)	≥1.5	>1.5
Platelets (× 10^9/L)	>100	>100
Hb (g/dL)	>11 (untransfused)	Not stated
Bone marrow lymphs (%)	<30; no nodules	Normal, allowing nodules or focal infiltrates
PR		
Physical exam (nodes, and/or liver, spleen)	≥50% decrease	Downshift in stage
Plus ≥1 of:		
Neutrophils (× 10^9/L)	≥1.5	
Platelets (× 10^9/L)	>100	
Hemoglobin (g/dL)	>11 or 50% improvement	
Duration of CR or PR	≥2 mo	Not stated
Progressive disease		Upshift in stage
Physical exam (nodes, liver, spleen)	≥50% increase or new	
Circulating lymphocytes	≥50% increase	
Other	Richter's syndrome	
Stable disease	All others	No change in stage

fore, the following lymphoid malignancies are specifically excluded from protocol studies directed at patients with B-CLL: T-CLL, prolymphocytic leukemia (B and T cell), hairy-cell leukemia and variant forms, splenic lymphoma with villous lymphocytes, large granular lymphocytosis, Sézary-cell leukemia, adult T-cell leukemia/lymphoma, and leukemic manifestations of non-Hodgkin's lymphomas, including follicular center-cell and mantle-cell lymphoma types.[12,15,17]

1.2. *Bone Marrow Examination*

A bone marrow aspirate and biopsy are generally not required to make the diagnosis of CLL. Nevertheless, CLL is a disease of the bone marrow, and it is appropriate to evaluate a major site of involvement. The aspirate smear must show ≥30% of all nucleated cells to be lymphoid. A bone marrow examination also provides useful prognostic information by determining whether there is diffuse or nondiffuse involvement,[18] and permits an assessment of the erythroid precursors and megakaryocytes.

1.3. *Immunophenotype*

As noted earlier, a thorough immunophenotypic profile of the malignant lymphocytes from the peripheral blood is necessary for the initial diagnosis of the patient with CLL.

1.4. *Molecular Biology/Cytogenetics*

Not only do cytogenetic analyses provide useful prognostic information, but they help identify potentially important nonrandom genetic alterations and oncogenes.[11,19-25] Sequential analysis of established genetic alterations may also be helpful in evaluating the evolution of the disease process. However, given the expense and limited availability of these studies, they should be restricted to a research setting in which to evaluate their potential prognostic and biologic importance.

2. Clinical Staging

We recognize that there are two somewhat different major staging methods that are currently in use throughout the world: the Rai system[26] and the Binet system.[27] In 1981, the IWCLL recommended that the two systems be integrated so that each of the Binet stages be subclassified with the Rai stage. However, the IWCLL-integrated system has not received widespread usage, and physicians continue to use either the Rai or Binet method in both patient care and in clinical trials. For clinicians using the Rai classification, we recommend the use of the modified version, which reduces the number of prognostic groups from five to three.[28] These two systems are outlined following.

2.1. *Rai System*

In the three-stage Rai system low risk encompasses Rai stage 0, with the clinical features of lymphocytosis in blood and bone marrow only. Intermediate risk encompasses stage

Table 2. Recommendations Regarding Evaluation and Monitoring of CLL Patients

Recommendation	General Practice*	Clinical Trial
Pretreatment evaluation		
History and physical	✓	✓
Examination of PBS	✓	✓
Immunophenotyping of PBLs	✓	✓
Bone marrow at diagnosis	+	✓
BM prior to therapy	✓¥	✓
Cytogenetic/molecular studies	X	•
CT scans, MRI, lymphangiogram gallium scan	X	X
Indications for treatment		
Treat with stage 0-1	X	•
Treat for active/progressive disease (newly dx)	✓	✓
Treat without active/progressive disease (newly dx)	X	•
Treat without active/progressive disease (relapsed/refractory)	X	•
Treat beyond maximum response	X	•
Response assessment		
CBC, differential	✓	✓
Bone marrow	+	✓
Phenotype	X	+
Cytogenetics/FISH	X	•

For purposes of this discussion, general practice is defined as the use of accepted treatment options for a CLL patient not enrolled on a clinical trial.

Abbreviations: ✓, always; X, not generally indicated; +, desirable; •, if a research question; ¥, if a study not performed recently, eg, at diagnosis; PBS, peripheral blood smear; PBLs, peripheral blood lymphocytes; dx, diagnosis; MRI, magnetic resonance imaging; FISH, fluorescence in situ hybridization.

I, with lymphocytosis and enlarged nodes, and stage II, with lymphocytosis plus splenomegaly and/or hepatomegaly (nodes positive or negative). High risk encompasses stage III, with lymphocytosis plus anemia, and stage IV, with lymphocytosis and thrombocytopenia.

2.2. *Binet Staging System*

Staging is based on the number of involved areas, and the level of hemoglobin (Hb) and platelet count. Whether significant adenopathy (>1 cm in diameter) is bilateral or unilateral is recorded.

Area of involvement considered for staging

(1) Head and neck, including the Wäldeyer ring (this counts as one area even if more than one group of nodes are enlarged).
(2) Axillae (involvement of both axillae counts as one area).
(3) Groins, including superficial femorals (involvement of both groins counts as one area).
(4) Palpable spleen.
(5) Palpable liver (clinically enlarged).

Stage A. Hb ≥10 g/dL and platelets ≥100 × 10^9/L and up to two of the above involved.

Stage B. Hb ≥10 g/dL and platelets ≥100 × 10^9/L and organomegaly greater than that defined for stage A, ie, three or more areas of nodal or organ enlargement.

Stage C. All patients, irrespective of organomegaly in whom Hb less than 10 g/dL and/or platelets less than 100 × 10^9/L.

3. Eligibility Criteria for Clinical Trials

3.1. *Clinical Stage*

The stage of CLL eligible for a clinical trial should reflect the therapeutic objectives, anticipated toxicities, and desired end results for each study. For example, a phase I study should involve only patients in advanced stages (Rai high risk, poor prognosis), while phase II and, particularly, phase III studies may also include patients in the intermediate-risk group. Decisions will be based on pilot data from phase I and early phase II trials with the particular agent or regimen. Patients with Rai stage 0 disease should generally not be entered into clinical trials. Other requirements for eligibility for clinical trials with respect to age, clinical stage, performance status, organ function, and status of disease activity should be defined for each study.

3.2. *Performance Status*

For phase I clinical trials, only patients with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 to 2 should be eligible. For phase II and III clinical trials, patients with PS 0 to 3 may be eligible; however, these limits may be individualized on the basis of the drugs or therapies being tested. For trials in which it appears reasonable to include patients with PS 3, yet where there is a concern over the potential toxicities of an agent or therapy, it may be advisable to initially start those patients at a lower dose of the treatment (eg, 50% reduction) and to gradually increase the dose over subsequent courses to the standard dose, if the treatment is well tolerated and toxicity is within an acceptable range. This approach should be individualized for each relevant protocol and may not be appropriate for some therapies (eg, high-dose therapy with stem-cell support).

3.3. *Organ Function Eligibility for Clinical Trials*

Most chemotherapy agents possess the potential for toxicity to the liver, kidneys, heart, lungs, central or peripheral nervous system, or other organ systems. Therefore, organ function requirements must be guided by the known toxicities of each drug based on observations from animal studies and previous therapeutic trials. Normal function for organs for which there is a well-recognized, specific toxicity must be required. Otherwise, as a general principle:

3.3.1. Baseline liver enzymes (ie, transaminase levels) should be no worse than 1.5 times the upper range of normal values. Serum bilirubin concentration should be ≤2.0 mg/dL, unless resulting from documented hemolysis.

3.3.2. Baseline renal function (ie, blood urea nitrogen [BUN], creatinine) should be no worse than 1.5 times the upper range of normal values.

3.3.3. Baseline requirements for other studies (eg, systolic ejection fraction, pulmonary function tests) should be decided individually for each study.

3.4. *Infection Status*

3.4.1. Patients with active infections requiring systemic antibiotics should be excluded from B-CLL clinical trials until resolution of infection.

3.4.2. Patients who are human immunodeficiency virus

(HIV)-positive should be excluded because of their poor tolerance to chemotherapy and the potential risks from the immunosuppressive effects of new agents such as fludarabine.[29]

3.5. *Second Malignancies*

Patients with a second malignancy, other than non–basal-cell carcinoma of the skin or in situ carcinoma of the cervix, should not be entered onto a CLL clinical trial unless the tumor was treated with curative intent at least 2 years previously.

3.6. *Required Pretreatment Evaluation*

As already noted, the parameters that are considered necessary for a complete pretreatment evaluation differ whether the patient is being treated in a general practice setting or on a clinical research protocol (Table 2). In general, and where feasible, these studies should be quantified within 48 hours of placing a patient on a treatment protocol (except for bone marrow aspirate and biopsy) (see later), and computed tomography (CT) scans (see later). They should also be repeated at appropriate intervals to assess the maximum response to therapy.

3.61. Complete blood cell count (CBC; white blood cell count, hemoglobin and hematocrit, platelet count) and differential, including both percent and absolute number of lymphocytes and prolymphocytes, and reticulocyte count.

3.62. Unilateral bone marrow aspirate and biopsy should be performed within 2 weeks prior to entering the study, unless a previous diagnostic specimen was diffusely involved and there has been no intervening systemic therapy. It is preferable to evaluate the bone marrow at diagnosis for prognostic purposes; however, it is mandatory in clinical trials, and highly desirable in clinical practice, to perform a unilateral bone marrow aspirate and biopsy prior to treatment to provide a baseline for further response assessment. If a repeat bone marrow is obtained, it should be reviewed along with the original diagnostic sample.

3.63. Lymph node evaluation

3.531. Physical examination should record the diameter, in two planes, of the largest palpable nodes in each of the following sites: cervical, axillary, supraclavicular, inguinal, and femoral.

3.632. Chest radiograph.

3.633. CT scans are generally not necessary in the initial evaluation of patients with CLL, but should only be performed if clinically indicated. A chest CT may be useful if the chest radiograph shows hilar adenopathy. These should be obtained within 2 weeks prior to entering the protocol.

3.634. Lymph node biopsy is generally not indicated, unless such tissue is necessary for companion scientific studies.

3.64. Liver and spleen size should be assessed by physical examination. CT scans should only be performed if clinically indicated or if part of a research question (see section 3.433).

3.65. Serum chemistries (eg, creatinine, bilirubin).

3.66. Assessment of PS (ECOG).

3.67. Baseline immunobiologic, cytogenetic, and molecular assessment for CLL trials (see Table 2, and earlier). Those that should be performed on all patients include serum immunoglobulin determination, including quantitative immunoglobulins and immunoelectrophoresis, direct and indirect antiglobulin (Coombs' test), and immunophenotypic evaluation of the B-cell clone (see earlier).

4. Indications for Treatment

4.1. *Primary Treatment Decisions*

Once the diagnosis of CLL has been made, the treating physician is faced with the decision of not only how to treat the patient, but when to initiate therapy. Criteria for initiating treatment may be quite different between clinical practice and clinical trial conduct. A subset of patients are considered as having smoldering CLL; they include those with Rai stage 0 (Binet A), with a nondiffuse pattern of bone marrow involvement, a serum Hb concentration $\geq$13.0 g/dL, peripheral blood lymphocytes less than 30×10^9/L, and a lymphocyte doubling time longer than 12 months.[13,14] Therapy should not be offered to these patients until they exhibit clear evidence of disease progression. Other newly diagnosed patients with early stage disease (Rai 0 to I, Binet A), should be monitored without therapy until evidence of disease progression. Studies from both the French Cooperative Group on CLL and the Cancer and Leukemia Group B (CALGB) in patients with early-stage disease confirm that early therapy of patients with early-stage disease does not prolong survival,[30,31] but may be associated with an increased frequency of fatal epithelial cancers.[30] However, these studies were conducted with alkylator-based regimens, and the potential benefit of earlier therapy using nucleoside analog therapy is an important research question.

Whereas most patients with Rai stages III and IV require treatment at presentation, many can still be monitored without therapy until they exhibit evidence of progressive or symptomatic disease.

Active disease should be clearly documented for protocol therapy. The following criteria must be met:

(1) A minimum of any one of the following disease-related symptoms must be present:
   (a) Weight loss $\geq$10% within the previous 6 months.
   (b) Extreme fatigue (ie, ECOG PS 2 or worse; cannot work or unable to perform usual activities).
   (c) Fevers of greater than 100.5°F for $\geq$2 weeks without evidence of infection.
   (d) Night sweats without evidence of infection.
(2) Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
(3) Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroid therapy
(4) Massive (ie, >6 cm below the left costal margin) or progressive splenomegaly
(5) Massive nodes or clusters (ie, >10 cm in longest diameter) or progressive lymphadenopathy
(6) Progressive lymphocytosis with an increase of >50% over a 2-month period, or an anticipated doubling time of less than 6 months
(7) Marked hypogammaglobulinemia or the development of a monoclonal protein in the absence of any of the above criteria for active disease is not sufficient for protocol therapy

Patients with CLL may present with a markedly elevated leukocyte count; however, the symptoms referable to leukocyte aggregates that develop in patients with acute leukemia rarely occur in patients with CLL. Therefore, the absolute lymphocyte count should not be used as the sole indicator for treatment, but should be included as a part of the total clinical picture, which includes the lymphocyte doubling time (see earlier).

4.2. *Second-Line Treatment Decisions*

Treatment of CLL is generally palliative in intent; therefore, patients who have relapsed may be followed without therapy until they experience disease-related symptoms or progressive disease, with deterioration of blood counts, discomfort from lymphadenopathy or hepatosplenomegaly, recurrent infections, or associated autoimmune disorders. A possible exception is allogeneic bone marrow transplantation. Recent data suggest that, in selected patients, allogeneic bone marrow transplantation or high-dose chemotherapy with autologous stem-cell support may be reasonable treatment options, particularly in the context of a clinical research protocol.[32-34]

The acceptable extent of prior therapy for protocol entry must be decided separately for each study.

(A) For all phase III therapeutic trials, it is recommended that only those patients who have not received previous cytotoxic or biological therapy be eligible. It is appropriate to include patients who have received previous corticosteroids if this is compatible with the therapeutic objectives of the trial. However, it may be necessary to analyze these previously treated patients as a separate group.

(B) For phase I and II studies, we recommend that no more than two types of prior therapy (eg, fludarabine, chlorambucil with or without prednisone) be allowed for entering patients. Certain trials may require previously untreated patients; this will be determined separately depending on the objectives of the study.

5. Definition of Response

Assessment of response should include a careful physical examination and evaluation of the peripheral blood and bone marrow. The response criteria in the original NCI-WG guidelines have been retained (Table 3).

5.1. Complete remission requires all of the following for a period of at least 2 months:

5.11. Absence of lymphadenopathy by physical examination and appropriate radiographic techniques.

5.12. No hepatomegaly or splenomegaly by physical examination, or appropriate radiographic techniques if in a clinical trial.

5.13. Absence of constitutional symptoms.

5.14. Normal CBC as exhibited by:

5.141. Polymorphonuclear leukocytes ≥ 1,500/μL.

5.142. Platelets > 100,000/μL.

5.143. Hemoglobin > 11.0 g/dL (untransfused).

5.15. Bone marrow aspirate and biopsy should be performed 2 months after clinical and laboratory results demonstrate that all of the requirements listed in 5.11 to 5.14 have been met to demonstrate that a CR has been achieved. The marrow sample must be at least normocellular for age, with less than 30% of nucleated cells being lymphocytes. Lymphoid nodules should be absent. If the bone marrow is hypocellular, a repeat determination should be made in 4 weeks. Samples should be re-reviewed in conjunction with the prior pathology.

Table 3. Grading Scale for Hematological Toxicity in CLL Studies

Decrease in Platelets* or Hb† (nadir) From Pretreatment value (%)	Grade‡	ANC/μL§ (nadir)
No change–		
10%	0	≥2,000
11%–24%	1	≥1,500 and <2,000
25%–49%	2	≥1,000 and <1,500
50%–74%	3	≥500 and <1,000
≥75%	4	<500

* If, at any level of decrease the platelet count is <20,000/μL, this will be considered grade 4 toxicity, unless a severe or life-threatening decrease in the initial platelet count (eg, ≤20,000/μL) was present pretreatment, in which case the patient is inevaluable for toxicity referable to platelet counts.

† Baseline and subsequent Hb determinations must be performed before any given transfusions.

‡ Grades: 1, mild; 2, moderate; 3, severe; 4, life-threatening; 5, fatal. Death occurring as a result of toxicity at any level of decrease from pretreatment will be recorded as grade V.

§ If the absolute neutrophil count (ANC) reaches less than 1,000/μL, it should be judged to be grade 3 toxicity. Other decreases in the white blood cell count, or in circulating granulocytes, are not to be considered, since a decrease in the white blood cell count is a desired therapeutic end point. A gradual decrease in granulocytes is not a reliable index in CLL for stepwise grading of toxicity. If the ANC was less than 1,000/μL prior to therapy, the patient is inevaluable for toxicity referable to the ANC.

5.16. For patients who fulfill all of the previous criteria for a CR, an abdominal CT scan may be performed to confirm this clinical and hematologic impression if clinically indicated or if required testing for a clinical research study.

5.2. PR is considered in a broad sense to enable the detection of agents with biological effect. To be considered a PR, the patient must exhibit 5.21 and 5.22 and/or 5.23 (if abnormal prior to therapy), as well as one or more of the remaining features for at least 2 months. In addition, the presence or absence of constitutional symptoms will also be recorded.

5.21. ≥50% decrease in peripheral blood lymphocyte count from the pretreatment baseline value.

5.22. ≥50% reduction in lymphadenopathy.

5.23. ≥50% reduction in the size of the liver and/or spleen.

5.24. Polymorphonuclear leukocytes ≥1,500/μL or 50% improvement over baseline.

5.25. Platelets >100,000/μL or 50% improvement over baseline.

5.26. Hemoglobin >11.0 g/dL or 50% improvement over baseline without transfusions.

5.27. In a subset of patients who are otherwise in a complete remission, bone marrow nodules can be identified histologically. It is, unfortunately, difficult with currently available techniques to determine the clonality of these nodules. The original NCI-WG guidelines suggested that patients with a CR and persistent nodules should be analyzed

carefully to compare their outcome relative to others who are more conventionally classified as a CR or PR.[1] Robertson et al[35] have since demonstrated that patients with a nodular CR had a shorter time to disease progression compared with patients with a CR. Therefore, nodular CRs should be reported separately from CRs, and should not be used to inflate the percentage of CRs. We recommend that they be referred to as nodular PRs (nPR) and included with the PRs.

5.28. A controversial issue is how best to categorize the response of patients who fulfill all the criteria for a CR, but who have a persistent anemia or thrombocytopenia apparently unrelated to disease activity and more likely the consequence of persistent drug toxicity. The long-term outcome of these patients may differ from the more routine complete responders. Therefore, these patients should not be considered CRs or a separate response category, but should be considered PRs. However, they should be monitored prospectively to better characterize their outcome, and may be described within the context of results of clinical trials.

5.3. Progressive disease will be characterized by at least one of the following:

5.31. ≥50% increase in the sum of the products of at least two lymph nodes on two consecutive determinations 2 weeks apart (at least one node must be ≥2 cm); appearance of new palpable lymph nodes.

5.32. ≥50% increase in the size of the liver and/or spleen as determined by measurement below the respective costal margin; appearance of palpable hepatomegaly or splenomegaly, which was not previously present.

5.33. ≥50% increase in the absolute number of circulating lymphocytes to at least 5,000/μL.

5.34. Transformation to a more aggressive histology (eg, Richter's syndrome or PLL with >55% prolymphocytes).

5.35. In the absence of progression, as defined earlier, the presence of a ≥2 g/dL decrease in Hb, or ≥50% decrease in platelet count, and/or absolute granulocyte count will not exclude a patient from continuing the study. Each protocol will define the amount of drug(s) to be administered with such hematological parameters. Bone marrow aspirate and biopsy are strongly encouraged to better define the cause of the suppressed counts.

5.4. Patients who have not achieved a CR or a PR, or who have not exhibited PD, will be considered to have stable disease.

5.5. Responses that should be considered clinically beneficial include CR, nPR and PR; all others, eg, stable disease, nonresponse, progressive disease, and death from any cause, should be rated as a treatment failure.

5.6. Because current criteria for response are arbitrary and often not validated by prospective studies, alternative criteria may also be evaluated; however, to ensure comparability with other studies, these should be studied within the framework of the current schema and be well defined, with adequate rationale. Should such a schema be determined to have important clinical relevance following prospective evaluation, it will be considered for incorporation into criteria for future studies.

5.7. Duration of response should be measured from the time the patient has exhibited the features of maximum response until evidence of progressive disease. Survival duration should be measured from the time of entry onto the clinical trial.

6. Prognostic Factors Requiring Stratification

6.1. Previous treatment versus no previous treatment in studies for which prior therapy is allowed.

6.2. If more than one clinical stage is allowed, patients should be stratified for stage (eg, if intermediate and poor risk are eligible, intermediate *v* poor), depending on the nature of the study and the available patient resources.

6.3. *Application of New Prognostic Factors*

In the interval since the initial publication of the guidelines, several modifications have been recommended.

6.31. Decrease in lymphocyte count: In several recent studies, a decrease in the peripheral blood lymphocyte count has been used as the primary index of response.[36] Although this parameter may identify a therapy that has lymphocytotoxic activity, there is no evidence that it has long-term clinical implications. It has, therefore, not been incorporated into the current response criteria.

6.32. Immunobiological assessment

6.321. Quantification of the serum immunoglobulin concentration in responders is recommended at the time of maximal clinical response, but it is not an established indicator of response.

6.322. Repeat immunophenotyping at the time of a response is not part of standard practice. Moreover, progression of disease after a CR should not be based purely on the basis of a small number of clonal cells identified using flow cytometric determinations.

6.323. In the clinical trials setting, not only should the peripheral blood smear and bone marrow aspirate and biopsy be carefully examined, but immunophenotype, cytogenetics, (including fluorescent in situ hybridization [FISH]), and molecular biologic studies provide important data and should be performed at diagnosis, at the time of maximal response, and at recurrence if part of a research question.

6.324. Serum β_2-microglobulin is recommended as an inexpensive prognostic marker.[37]

6.325. Other optional studies that may be of interest include markers of B-cell proliferation such as Ki-67, which might identify alterations in the malignant cell population, soluble CD23, adhesion molecules, or molecular analysis of specific genes (eg, oncogenes, tumor-suppressor genes). These scientific parameters that assess the biology of the malignant clone may help us to identify new therapeutic strategies.

6.33. Minimal residual disease: The optimal approach to the patient with minimal residual disease remains another important research issue. Careful assessment for minimal residual disease as determined by flow cytometry, cytogenetics, or similar studies is not indicated outside of a research study at the time of CR and at recurrence. Additional treatment decisions on the basis of minimal residual disease remains an issue for clinical investigation.

7. Assessment of Toxicity

An evaluation of potential treatment-induced toxicity in patients with advanced malignancies may be quite difficult, requiring careful consideration of both the manifestations of

the underlying disease, as well as adverse reactions to the therapies under study. Moreover, some of the conventional criteria for toxicity are not applicable to studies involving patients with hematological malignancies in general, or CLL in particular. An example is hematological toxicity; patients with advanced CLL may exhibit a deterioration in blood counts, which may represent either treatment-related toxicity or progressive bone marrow failure from the disease itself. This discrimination may become increasingly difficult as new agents are tested earlier in their development at a point where the complete spectrum of their toxicities has not yet been elaborated.

A few guidelines are presented recognizing that evaluation methods will be determined to a large extent within the therapy involved.

7.1. *Hematological Toxicity*

As is the case with virtually all of the hematological malignancies, an evaluation of hematological toxicity in patients with CLL must consider the high frequency of hematological compromise at the initiation of therapy. Therefore, the standard criteria used for solid tumors cannot be applied directly; many patients would be considered to have grade II to IV hematological toxicity at presentation.

Also, in the past, the peripheral blood neutrophil level has rarely been used as a criterion for dose modification since these values were felt to be unreliable in CLL. However, the increasing use of more effective therapeutic agents, particularly those with neutropenia as a dose-limiting toxicity (eg, nucleoside analogs), has resulted in clinically significant myelosuppression. Therefore, we have proposed a new dose-modification scheme for quantifying hematological deterioration in patients with CLL, which includes alterations in the dose of myelosuppressive agents based on the absolute neutrophil count (Table 3).

7.2. *Infectious Complications*

In CLL, as with many other hematological malignancies, it may be difficult to distinguish between the occurrence of infections related to the disease itself or to the consequences of therapy. However, such an analysis is of value when comparing the results of various treatments, particularly with immunosuppressive agents such as the nucleoside analogs.[29] The etiology of the infection should be reported and categorized as bacterial, viral, or fungal, and proven or probable. The severity of infections should be quantified as minor (requiring either oral antimicrobial therapy or symptomatic care alone), major (requiring hospitalization and systemic antimicrobial therapy), or fatal (death as a result of the infection).

7.3. *Nonhematological Toxicities*

Other nonhematological toxicities should be graded according to the NCI Common Toxicity Criteria.[38]

8. Reporting of Clinical Response Data

Clear and careful reporting of data is an essential part of any clinical trial. In clinical studies involving previously treated patients, patients who are relapsed or refractory should be clearly distinguished. Relapse is defined as a patient who has previously achieved the clinicopathologic criteria to be classified as a CR or PR, but, after a period of ≥6 months, demonstrated evidence of disease progression (Table 1). For those patients who have relapsed, it is also useful to describe the quality and duration of their prior response. Refractory disease refers to the clinical situation in which a patient fails to achieve at least a PR or progresses while on therapy.

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EXHIBIT C

American Journal of Hematology 40:259–263 (1992)

Reduced Expression of CD20 Antigen as a Characteristic Marker for Chronic Lymphocytic Leukemia

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The surface antigens expressed by the cells of chronic lymphocytic leukemia (CLL) are well known. Most CLL are monoclonal B-cell lymphoproliferative disorders characterized by the coexpression of B-cell antigens and CD5, an antigen present predominantly on T cells. Very little attention, however, has been paid to the quantitative characteristics of the expression of B-cell antigens in CLL. In this study, we used flow cytometry to analyze the expression of CD20, a well-known B-cell-associated antigen, in lymphocytes from 42 cases of CLL and its tissue counterpart, small lymphocytic lymphoma (SLL), and compared the results with results obtained from the analysis of 21 follicular lymphomas, 20 hyperplastic reactive nodes, and 26 samples of normal peripheral blood. The intensity of CD20 expression in the CLL/SLL cells was significantly lower than that of B cells in the other categories. This antigen expression abnormality does not appear to be a universal phenomenon in CLL/SLL, since CD19, another pan-B antigen, was expressed in CLL/SLL at levels higher than those in follicular lymphomas and comparable to those in reactive lymph nodes. These results indicate that the low CD20 expression can be used as a marker for CLL/SLL. The few cases exhibiting intense CD20 expression may represent a biologically different disease. CLL/SLL cells faintly expressing CD20 also show concomitant low CD5 expression in a manner not observed in normal CD5-expressing B cells. © 1992 Wiley-Liss, Inc.

Key words: B cells, CLL, flow cytometry, fluorescence, lymphomas

INTRODUCTION

Chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) are clonal proliferation of small lymphocytes involving peripheral blood, bone marrow, lymph nodes, spleen, and other organs. During the 1980s, major immunological advances and the introduction of monoclonal antibodies led to numerous studies aimed at characterizing the surface antigens expressed by CLL cells [1–4]. The majority of these studies focused on the presence or absence of expression of surface antigens on the leukemic cells, and very few considered the antigenic density as an important biological characteristic of the neoplastic cells [5]. In this study, we analyzed the expression of a variety of cell surface antigens on CLL and SLL cells by flow cytometry and specifically quantitated the intensity of the expression of CD20, a B-cell-associated antigen [6]. The results were compared with those of low-grade follicular lymphomas and of B cells from hyperplastic reactive lymph nodes and normal peripheral blood.

MATERIALS AND METHODS

Samples

Samples were obtained from materials submitted to us for diagnostic purposes. They included 36 peripheral blood specimens from patients with CLL, six lymph node biopsies from patients with SLL, 21 lymph node biopsies

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from patients with low-grade follicular lymphomas (FL), and 20 hyperplastic reactive nodes (RN) submitted to rule out lymphoma. Normal peripheral blood (NPB) specimens were obtained from 26 healthy volunteers (aged 25–65 years). The diagnosis of these samples was based on hematological, morphological, and immunological criteria [7,8]. All cases diagnosed as CLL had increased number of circulating mature-looking lymphocytes, which labeled as B cells coexpressing CD5. These cells expressed either no detectable surface immunoglobulins or a single immunoglobulin light chain.

Tissue Processing

A portion of each lymph node was fixed in 10% buffered formalin and processed for routine histological examination. Single cell suspensions from lymph nodes were prepared as described elsewhere [9]. Peripheral blood mononuclear cells were obtained by centrifugation through a ficoll hypaque gradient. Peripheral smears were prepared and stained by the Wright Giemsa method for routine morphological examination.

Immunofluorescence Staining

Direct immunofluorescence staining with monoclonal or polyclonal antibodies was performed as described previously [9]. Briefly, antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) were placed at appropriate dilutions in the wells of microtiter plates [10]. One hundred twenty thousand cells were placed in each of the wells, incubated for 15 mins on ice, and washed in buffered saline solution twice. Antibodies in this study included monoclonal reagents against CD2 (Leu 5B), CD3 (Leu 4), CD4 (Leu 3A), CD5 (Leu 1), CD7 (Leu 9), CD8 (Leu 2A), CD10 (CALLA), CD19 (Leu 12), CD20 (Leu 16), and HLA-DR (HLA-DR) and polyclonal antibodies against individual immunoglobulin light chains. Controls included FITC- and PE-conjugated isotype-matched normal mouse IgG. All monoclonal antibodies were obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA), and the polyclonal antibodies were from either Caltag Laboratories (South San Francisco, CA) or Kallestad Laboratories (Austin, TX). Dual-color (FITC/PE) immunofluorescence combinations included antibodies against CD19/kappa, CD19/lambda, and CD20/CD5. According to the manufacturer, the various lots of antibodies against CD20 used in this study were conjugated with FITC at F/P ratios ranging from 4.7 to 4.9, and the variation in mean fluorescence intensity of normal blood B cells stained with the various lots of reagents did not exceed 4%.

Data Analysis

Data were collected on a Facscan flow cytometer (Becton Dickinson Immunocytometry Systems). The fluorescence data were collected with logarithmic amplification, and Lysys software (Becton Dickinson Immunocytometry Systems) was used to convert the logarithmic data into linear-equivalent fluorescence values. Ten thousand cells were collected in each sample, and data were stored in list mode. Expression of the various antigens was visually determined on forward light scatter/fluorescence intensity bivariate plots, by comparing binding of specific antibody with that of the isotype-matched IgG control. Intensity of CD20 and CD19 expression of lymphocytes was calculated by dividing the peak value of the linearized fluorescence of the positive distribution by that of the isotype-matched IgG control. The assessment of immunoglobulin light chain expression was performed by analyzing immunoglobulin light chain distributions of the CD19-expressing cells only.

TABLE I. Intensity of Expression of CD20 on B cells

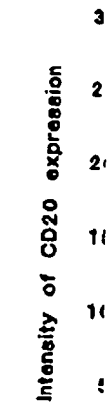
Diagnosis[a] (No. of cases)	CD20 intensity (median)	*P* value[b]	CD19 intensity (median)	*P* value[b]
CLL/SLL (42)	13.7		16.4[c]	
FL (21)	84	<0.0005	10	<0.005
RN (20)	67.9	<0.0005	19	ns
NPB (26)	90.4	<0.0005	ND[d]	—

[a]CLL, chronic lymphocytic leukemia; SLL, small lymphocytic lymphoma; FL, low grade follicular lymphoma; RN, reactive nodal hyperplasia; NPB, normal peripheral blood.
[b]Values were calculated by comparing cases from each group to those of CLL/SLL using the Kruskal-Wallis two-sample test. ns, Not significant.
[c]Only 41 cases analyzed.
[d]ND, not done.

RESULTS

In addition to B-cell antigens (CD19 and CD20) and CD5, CLL/SLL cells expressed HLA-DR but lacked expression of T-cell markers such as CD2, CD3, CD4, CD7, and CD8. No surface immunoglobulin was detected in two cases, and the rest showed expression of a single immunoglobulin light chain (28 kappa, 12 lambda), which was often faint. CD10 was not expressed in any of the CLL/SLL cases. All cases of FL demonstrated HLA-DR, CD19, and CD20 but lacked CD5 expression. FL also showed single immunoglobulin light chain expression (15 kappa, 6 lambda), and CD10 was present in 76% of the cases. In contrast, only 15% (3/20) of the RN expressed CD10. None of the RN demonstrated a restricted light chain expression.

The intensity of CD20 expression in the 42 cases of CLL/SLL is shown in Table and Fig. 1. The median fluorescence intensity of CD20 in CLL/SLL was 13.7 (range 1.5–174), whereas the median values in FL, RN, and NPB were 84 (range 12–298.6), 67.9 (range 16–174), and 90.4 (range 40–199), respectively. The differ-



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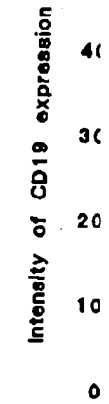

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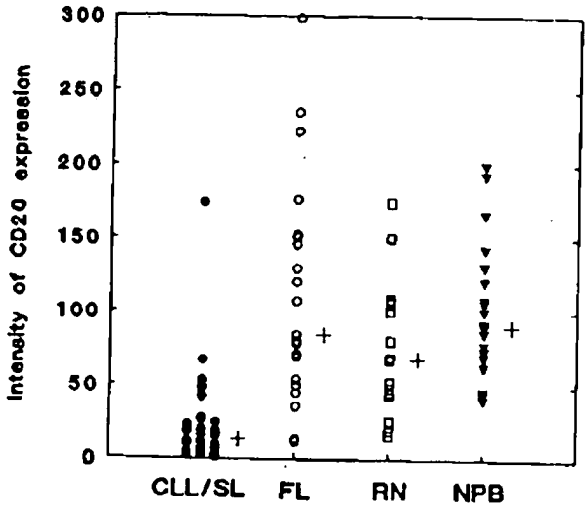

Fig. 1. Intensity of CD20 expression in 42 cases of chronic lymphocytic leukemia (CLL), 21 cases of follicular lymphoma (FL), 20 cases of reactive lymph nodes (RN) and 26 samples of normal peripheral blood (NPB). Crosses indicate median values.

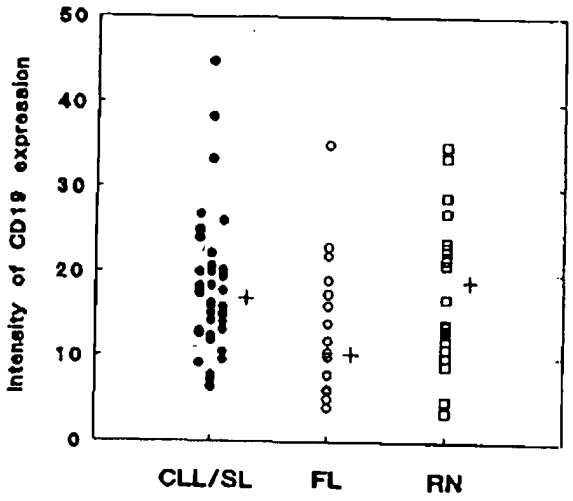

Fig. 2. Intensity of CD19 expression in 41 cases of chronic lymphocytic leukemia (CLL), 21 cases of follicular lymphoma (FL) and 20 cases of reactive lymph nodes (RN). Crosses indicate median values.

ences between CD20 intensity of expression in CLL/SLL and that of each of the other groups were highly statistically significant (Kruskal-Wallis 2-sample test, $P < 0.0005$). The median fluorescence intensity of CD19 was 16.4 (range 6.4–44.8) for CLL/SLL, 10 (range 4–35) for FL, and 19 (range 3.5–34.9) for RN. In this study, CD19 antibodies were not tested in NPB. There was no significant difference between the intensity of CD19 expression in CLL/SLL cells and in B cells of RN. However, CD19 expression in CLL/SLL cells was more intense than in B cells of FL ($P < 0.005$) (Fig. 2).

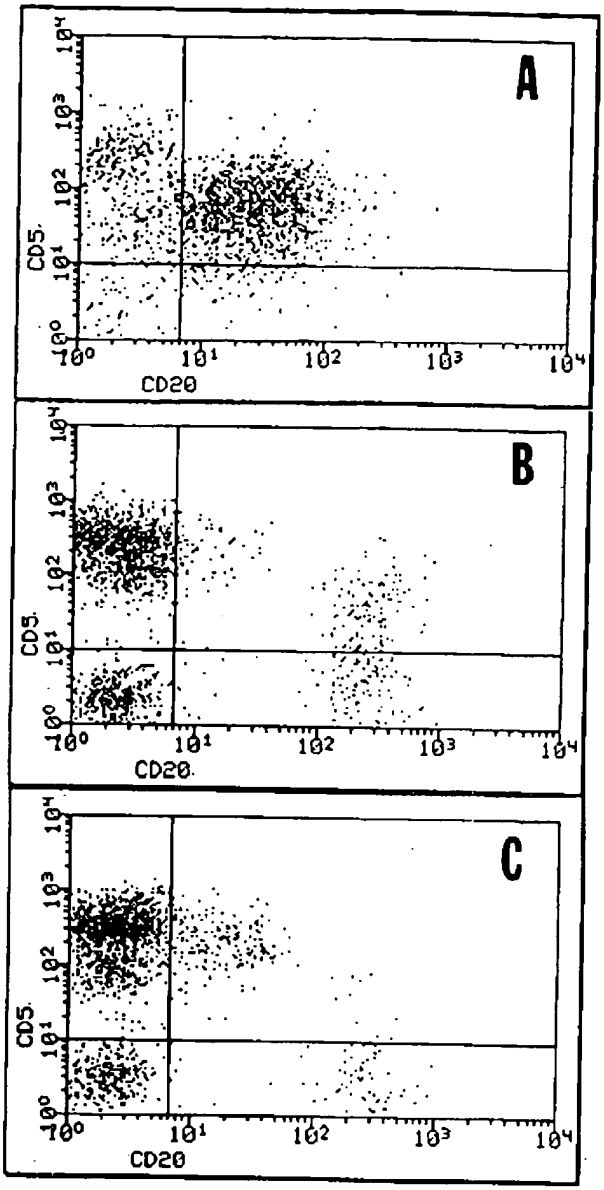

Fig. 3. Dual color analysis of CD20 and CD5 expression in a case of chronic lymphocytic leukemia (A) and normal peripheral blood (B, C). Cells were simultaneously exposed to FITC-labelled anti-CD20 and PE-labelled anti-CD5. The majority of cells in A exhibit faint CD20 and CD5. No cells with similar intensity of expression are seen in B and C. Normal blood lymphocytes coexpressing CD20 and CD5 demonstrate either normal CD20 and faint CD5 expression (B) or normal CD5 and faint CD20 expression (C).

Simultaneous dual-color correlated immunofluorescence analysis was performed in NPB to quantitate CD20 and CD5 coexpressing cells-which may represent putative normal counterparts of CLL cells. This analysis revealed the presence of two discrete small subpopulations of lymphocytes coexpressing CD5 and CD20. One of the subpopulations expressed CD20 antigen with a fluores-

cence intensity similar to that of normal B cells but with less CD5 expression than normal T cells (Fig. 3B). The other subpopulation showed exactly the opposite pattern, with a relatively bright CD5 expression (similar to normal T cells) and a relatively dimmer CD20 expression in comparison to the normal B cells (Fig. 3C). Because of their relatively low numbers and the faint expression of either antigen, a precise quantitation of these subpopulations was not possible. In all of our CLL/SL cases, CD5 was expressed at lower levels than in normal T cells (data not shown). Thus, with the exception of those few cases when CD20 was detected in normal amounts, CLL/SL cells did not coexpress CD20 and CD5 antigens in the same manner as NPB lymphocytes.

DISCUSSION

The immunophenotypic analysis of CLL/SLL, FL, RN, and NPB performed in our laboratory confirms in general the data published in the literature [1–4,11,12]. However, our study demonstrates the unique feature of faint intensity of CD20 expression on CLL/SLL cells. CD20 intensity expression on neoplastic B cells has not been thoroughly examined previously. We are aware of four publications in which this analysis was specifically addressed [5,13–15]. Our results are similar to those of Cossman et al. [13], who found that the cells of ten SLL cases had less CD20 expression than either follicular center cell lymphoma or intermediately differentiated lymphocytic lymphomas. Likewise, Marti et al. [5] found a reduced expression of CD20 reagents in five cases of CLL. On the other hand, our findings are somewhat different from those of Freedman et al. [14], who found that CD20 was expressed strongly on CLL cells. We also cannot confirm the high frequency of bright CD20 expression in CLL described by Maddy et al. [15], who divided CLL cases into two approximately equal groups on the basis of the intensity of expression of high- and low-molecular-weight leukocyte common antigen (LCA) and found high expression of CD20, CD21, and CD22 in the subgroup with denser expression of high-molecular-weight LCA. These discrepancies may be due, in part, to different staining procedures, to dissimilar fluorochromes, or to the cell separation procedures used.

One may argue that, although in our study antibodies were used in saturating amounts, CLL samples contain many more B cells than samples of normal blood. Thus the decreased staining with CD20 antibody could be due to insufficient reagent available in the preparations to saturate the B-CLL cells. This is very unlikely, since the low CD20 expression is not observed in other B-cell lymphoproliferative disorders containing the same relative number of B cells, and residual CD5-negative normal B cells sometimes present in CLL/SL samples express normal levels of CD20 (data not shown).

It is important to emphasize that the values of fluorescence intensity reported in this paper apply only to the cases studied in our laboratory and could not be directly compared with results obtained with different reagents and/or other instruments. The results of the measurements could be standardized if they were expressed as antibody binding sites per cell. This would require the use of standard particles to measure quantitatively both the molecules of equivalent fluorochrome and the average fluorescence intensity per antibody molecule for each reagent [16].

In the past, attempts to correlate immunophenotypic analysis with prognosis in CLL were unsuccessful [17–19]. However, it should be noted that these studies dealt primarily with presence or absence of surface antigens, with little emphasis on the intensity of expression. In our series, very few cases of CLL show intense CD20 expression. We cannot determine at this time the clinical relevance of this observation, but the density of surface CD20 may indicate a biologically relevant phenomenon in CLL and may represent a variable of prognostic value. In fact, we frequently observed intense CD20 expression in cases of prolymphocytic leukemia studied in our laboratory (data not shown).

Many observers have correlated phenotypic markers of B-CLL with those expressed at various stages of maturation of normal B cells. Cossman et al. [13] hypothesized that the tumor cells in SLL are more differentiated and closer to plasma cells than those of follicular and intermediately differentiated lymphoma cells. Indeed, the notion that CLL/SLL cells are at a late stage of differentiation is substantiated by in vitro studies showing that activation of B cells leads to a characteristic phenotype resembling that of B-CLL [14]. Although we did not study the maturation sequence of neoplastic B cells, we show that there is a decrease in the intensity of CD20 in B cells of RN compared with NPB, suggesting that stimulation/activation and perhaps further differentiation of B cells may be associated with a decrease in CD20 expression. It is believed that CD20 functions as a calcium channel (T. Tedder, personal communication) and that it regulates a step in B cell activation that is required for further differentiation [20]. This suggests that B cells at different stages of differentiation may express different levels of CD20. However, it is uncertain whether the surface antigens of CLL/SLL cells reflect a unique stage of normal B cell differentiation or represent an abnormal phenotype due to neoplastic transformation.

The few B cells that normally coexpress CD5 and CD20 may expand in autoimmune disorders [21–23] and may be the target for neoplastic transformation in CLL. The dual-color analysis performed in our samples in healthy donors confirmed the presence of these cells in small percentages. However, we found two populations of such cells, and, judging by their antigenic density, we

believe that neither of the two coexpresses CD5 and CD20 in a manner comparable to the surface characteristics of the typical CLL/SLL.

In summary, our data indicate that dim CD20 expression is a unique feature of CLL/SLL cells. The small number of CLL/SLL cases in which cells show intense expression of CD20 antigens may represent a biologically or even clinically different disorder. This group will require further study. Immunophenotypically, the CD5-positive B cells present in NPB do not resemble CLL/SLL cells.

Note Added in Proof

After submission of this manuscript, we became aware of a study in press in NY Acad Sci entitled "CD20 and CD5 Expression in B-Chronic Lymphocytic Leukemia (B-CLL)" by G.E. Marti, G. Faguet, P. Bertin, J. Agee, G. Washington, S. Ruiz, P. Carter, V. Zenger, R. Vogt, and P. Noguchi. Using a quantitative approach, these authors also found significantly less expression of CD20 on B-CLL cells than in normal B-cells from peripheral blood. They also confirmed the lower expression of CD5 on the leukemic cells in comparison to normal T-cells.

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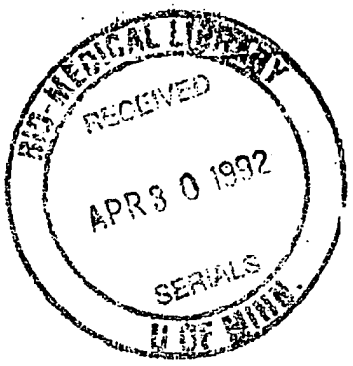

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EXHIBIT D

Chronic Lymphocytic Leukemia

83

Kanti R. Rai and Dilip V. Patel

INTRODUCTION

Chronic lymphocytic leukemia (CLL) as a distinct clinical entity was first identified in 1903 by Turk,[1] who gave us not only criteria for its diagnosis but also the features that distinguish it from lymphomas. In 1924 Minot and Isaacs[2] presented a detailed clinical description of CLL. Although several physicians studied this disease in the ensuing decades, it was not until 1966–1967 that the pathophysiology of CLL was fully explained. Galton[3] and Dameshek[4] independently but virtually simultaneously suggested that the main characteristic of CLL is a progressive accumulation of functionally incompetent, long-lived lymphocytes.

EPIDEMIOLOGY

CLL is the most common form of leukemia in the Western Hemisphere, accounting for about 25–30% of all leukemias. Approximately 10,000 new cases are diagnosed every year in the United States.[5] Characteristically, CLL is a disease of advancing age, the incidence being >20 per 100,000 persons >60 years of age.[6] However, the disease is being diagnosed in increasing frequency among younger age groups and is no longer considered unusual even in patients 35 years of age. The median age at diagnosis is 55 years. The incidence of CLL is higher among men, with the male/female ratio being nearly 2:1. CLL is rarely seen in Japan, China, and other Asian countries. The reason for this wide disparity in incidence of CLL in different parts of the world remains unknown. More than 95% of patients have a B-cell phenotype; T-cell CLL is a rare disease, accounting for only 2–5% of all cases. Unless otherwise specified, most descriptions of CLL pertain to B-cell disease.

GENETIC ASPECTS

CLL is an acquired disorder. There is one report in the literature of CLL occurring in twin sisters who were monozygous, but not identical. Immunoglobulin gene rearrangements in the CLL cells of these twins were found to differ from each other.[7] Relatives of CLL patients have an increased frequency of CLL, other B-cell malignancies, and autoimmune disorders.[8–11] The risk of developing CLL among first-degree relatives of patients with CLL is higher than expected. Consanguinity and chromosomal abnormalities have been proposed as possible explanations. No HLA haplotype has been found to be consistently associated with CLL.[12]

ETIOLOGY AND ONCOGENESIS

The etiology of B-CLL remains unknown. No causal relationship has been found with exposure to radiation, chemicals, and alkylating agents. Human T-cell leukemia/lymphoma virus 1 (HTLV-1) is known to cause adult T-cell leukemia. Although retroviruses and DNA viruses such as HTLV-1 and Epstein-Barr virus (EBV) are not considered to be etiologic agents for CLL, Mann et al.[13] have observed two patients with B-CLL who were HTLV-1 seropositive, but the virus was not present in the cellular genome.

In CLL there is a progressive accumulation of the leukemic lymphocytes, with no increase in their rate of proliferation. Lymphocytes in CLL are known to be long-lived. The early observations of Galton[3] and Dameshek[4] suggested that CLL lymphocytes are long-lived because they are functionally incompetent. The still unfolding story of the proto-oncogene *bcl-2*, however, indicates that at least in some cases of CLL the explanation for lymphocyte longevity may be the inhibition of programmed cell death (apoptosis). It was observed, initially in follicular lymphoma, that there is an association between the rearrangement of *bcl-2* and t(14;18) chromosomal translocation. *bcl-2* is known to interfere with apoptosis.

Early studies in CLL showed that only about 10% of patients had rearrangement of *bcl-2*.[14,15] However, subsequent studies have revealed that there are alternative mechanisms that result in accumulation of high levels of bcl-2 protein in CLL cells, which are independent of *bcl-2* rearrangements.[16] One possible mechanism is DNA-hypomethylation. Hanada et al.[16] demonstrated DNA hypomethylation in a relatively selected region of the *bcl-2* gene in 20 of 20 CLL cases studied. These data favor the explanation that an overexpression of bcl-2 protein, with its known ability to interfere with apoptosis, results in the long life of CLL lymphocytes.

GROWTH AND DIFFERENTIATION

Leukemic cells in CLL are known to be "arrested" in the G_0 phase of the cell cycle with a relatively rare cell in the peripheral blood showing evidence of being in the active proliferative cycle.[17,18] CLL cells, arrested in the late stages of B-cell differentiation, reveal certain characteristics in vitro that may explain at least some aspects of the pathophysiology of this disease. When cultured[19] without cytokines and mitogens, in vitro, CLL cells die rapidly by apoptosis. It has been demonstrated that CLL cells lose bcl-2 protein during culture.[20] Addition of interleukin (IL)-4[21] or interferon (IFN)-γ[22] to the cultures inhibits the cell death by apoptosis, an observation correlated with a simultaneous increased expression of bcl-2 protein in IL-4-treated B-CLL cells.[21] When CLL cells are cultured in the presence of B-cell mitogens or the phorbol ester TPA (12-0-tetradecanoylphorbol-13-acetate), they are capable of plasmacytoid differentiation, suggesting that these cells are not "frozen" at an intermediate stage in the B-cell differentiation pathway. The process of stimulation of differentiation of CLL cells, however, is complex and requires all the co-stimulatory factors necessary for normal B cells undergoing differentiation in vitro, including various cytokine producing non-neoplastic T cells.[23]

CHROMOSOMAL ABNORMALITIES

CLL lymphocytes are resting cells in G_0 and in vitro have no spontaneous mitoses. Until the recent introduction of B-cell mitogens, cytogenetic studies were not possible in CLL be-

cause metaphases were only rarely inducible. In the past decade, however, several laboratories have been successful in performing cytogenetic studies in CLL following the availability of a battery of B-cell mitogens, including lipopolysaccharide, TPA, cytochalasin B, pokeweed mitogen, and EBV supernate, which induce readable metaphases in a large proportion of cases. Juliusson and Gahrton[24] in an update on the cytogenetic data pooled by the International Working Party on Chromosomes in CLL and from additional data from the published literature, have provided a detailed status report on this subject. In this collected series[24] of G-banded metaphase analysis of 1,244 cases of CLL, clonal chromosomal abnormalities were seen in 43% (533 cases), and trisomy 12 was the most frequently observed abnormality, occuring in about 15% of all patients studied and about one-third of all cases with clonal abnormalities. Structural abnormalities involving the long arm of chromosome 13 accounted for 20% of cases with clonal abnormalities, and other, less frequently occurring structural abnormalities involved the long arms of chromosomes 6 and 14.[24]

Although structural abnormalities of the long arm of chromosome 13 involved different breakpoints, most consisted of deletion of band 13q14, the site of retinoblastoma (Rb) suppressor gene. The Rb gene product, however, is normally expressed in patients with 13q14 deletion,[25,26] perhaps because the other allele has a normal Rb gene.

Structural abnormalities involving chromosome 14 is of particular interest because this chromosome is the site for the genes for immunoglobulin heavy chain at band q32 at the distal end of the long arm. Clonal abnormalities in B-cell malignancies sometimes involve 14q32, including t(11;14) (q13;q32), which juxtaposes the *bcl*-1 gene[27,28] and the immunoglobulin heavy chain gene.

Among the cases with chromosomal abnormalities, more than one-half are constituted by single abnormalities and complex abnormalities are seen in 10–15%.[24]

Fluorescence in situ hybridization (FISH) with a chromosome 12-specific α-centromeric probe has enabled a study of numerical abnormalities of this chromosome in interphase cells in CLL, thereby overcoming the problem of inconsistency in inducing mitoses by banding techniques that require readable metaphases.[29] Using the FISH technique, Que et al.[30] noted trisomy 12 in 21 of 183 (11.5%) cases of CLL, whereas the conventional cytogenetic techniques could detect trisomy 12 in only 15 of these 21 cases. It is likely that further studies with the FISH technique will be useful in establishing the exact incidence of trisomy 12 in CLL without the need to depend on obtaining adequate metaphase preparations. However, the need to identify chromosomal abnormalities other than trisomy 12 requires that both conventional cytogenetics and FISH are necessary. At this time, cytogentic studies are not performed in the routine clinical management of CLL—they remain in the research domain.

IMMUNOPHENOTYPIC PROFILE OF LYMPHOCYTES

B-Cells

The normal T-cell/B-cell ratio is reversed in CLL. In B-CLL, the B cells usually account for nearly 90% of all lymphocytes. There is a characteristic immunophenotypic profile of B-cells as shown in Table 83-1.[31–33] CLL B-cells express low surface density of immunoglobulins, usually IgM or IgM with IgD, which are monoclonal as revealed by expression of only one light chain, either κ or λ. These cells form rosettes with mouse erythrocytes. Intracytoplasmic immunoglobulins may be detectable in a few cases. Some B cells have receptors for Fc fragments, for IgG, and for complement (C3d). By using a wide range of monoclonal antibodies, it has been established that B cells of B-CLL stain positively with one pan-T-cell antibody Leu-1 (CD5),[34–36] while simultaneously also staining with at least one of the B-cell monoclonal antibodies B1 (CD20), B4 (CD19), and BA1 (CD24). These cells express HLA-DR, the MHC class II antigen. There is considerable degree of heterogeneity in the expression of CD25 (IL-2 receptor), CD23 (activation marker, low-affinity FcE receptor), and CD11c (β_2 integrin) and surface adhesion molecules CD54, CD58, and L-selectin.[31,32,37] The overall interpretation of this phenotypic expression is that CLL cells are relatively mature cells that are arrested at an intermediate stage in the pathway of B-cell differentiation.

Table 83-1. Immunophenotypic Pattern of B-CLL Cells

Surface immunoglobulin (sIg):
Usually IgM or IgM and IgD of low intensity (infrequently, the amount of sIg may be so low that the cell is read as sIg negative)
Mouse-erythrocytes rosetting
One or more of the following B-cell markers
CD19 (B4), CD20 (B1), CD24 (BA1), CD21 (C3dR), CD23 (activation marker)
Ia
CD5 (pan-T, Leu-1)
Heterogeneous with respect to
CD11c (β_2 integrin)
Surface adhesion molecules CD54, CD58, L-selectin, and CD25 (IL-2 receptor)

The CD5 positivity of B cells in CLL has become a subject of active investigation. A very small subset of normal B lymphocytes is known to be $CD5^+$.[34] It is not clear whether this normal $CD5^+$ B-cell subpopulation is the one that proliferates and accumulates in CLL.[38] Normal $CD5^+$ B cells express activation markers and >10% of these cells may be in active proliferative cycle, whereas only <1% of $CD5^+$ B cells in CLL are in active cycle[38] (Table 83-1). Investigations in this area, however, do not provide clear evidence that $CD5^+$ B cells constitute a separate B-cell lineage.[39]

T Cells and Natural Killer Cells

The absolute number of T cells in B-CLL may be normal, decreased, or increased according to the total lymphocyte count and percentage of T cells. There is usually a reversal of the normal T-helper (CD4)/T-suppressor (CD8) cell ratio.[40–42] The population of large granular lymphocytes (natural killer [NK] cells) is usually decreased.[38,41–44] The published work on the functional status of T cells and NK cells in CLL is contradictory and difficult to interpret.[39,45]

Autoimmune Complications

Autoimmune complications are known to occur frequently in CLL.[46] It has been suggested that $CD5^+$ B cells may play an important role in the production of IgM autoantibodies. An increase in the number of $CD5^+$ B cells has been reported in rheumatoid arthritis and other autoimmune diseases.[47] Autoimmune phenomena in CLL are often directed against hematopoietic cells. A positive direct antiglobulin test has been reported in ≤35% of CLL cases. Autoimmune hemolytic anemia may occur in 10–25% of cases at some time during the course of the disease.[39] In most cases the autoantibodies against erythrocytes are warm reactive and polyclonal[48] with or without red-cell-associated C_3b or C_3d. Immune thrombocytopenia occurs in about 2% of CLL cases. Pure red cell aplasia and autoantibodies against neutrophils are observed less frequently.

Table 83-2. Factors Contributing to Immunodeficiency in CLL

Reduced serum immunoglobulin
Reduced percentage of $CD4^+$ cells, increased percentage of $CD8^+$ cells (worsening with disease prognosis)
Reduced response to antigens and mitogens

(Modified from Foa,[45] with permission.)

Abnormalities in γ-Globulin Levels

Hypogammaglobulinemia is not an unusual feature of CLL. The levels of serum IgG, IgA, and IgM may all be markedly decreased or just one or two of the immunoglobulin classes may be involved. The pathogenesis of this complication is poorly understood but regulatory abnormalities of helper T, suppressor T, NK, and antibody-dependent cellular cytotoxicity (ADCC) cells may play a role. NK cells from CLL patients with hypogammaglobulinemia were found to cause a decrease in immunoglobulin secretion by normal B cells.[49] It is also possible that a decrease in or inhibition of normal B cells ($CD5^-$) results in hypogammaglobulinemia.[39] CLL patients tend to have defective specific antibody response to infection and to immunization.[50] Infections with encapsulated organisms as well as with gram-negative bacteria are recognized as the most frequent cause of morbidity and mortality in CLL.[50,51]

A monoclonal serum immunoglobulin spike (usually IgM) has been observed in 5% of cases with CLL. However, Deegan et al.,[52] using high-resolution agarose gel electrophoresis and immunofixation, observed a small amount of monoclonal protein in the serum and urine of 60% of CLL patients.[52]

A summary of the presently recognized factors that may play a role in the heterogeneous immunologic abnormalities in CLL is shown in Table 83-2. There are additional factors (such as reduced NK, ADCC, and lymphokine-activated killer cell activity, increased levels of soluble IL-2 receptors, and reduced IL-2 availability), but their role is far from clearly proven. The available data strongly suggest that the abnormalities of the T- and cytotoxic cell compartments represent a secondary event occurring during the course of CLL.[45]

CLINICAL MANIFESTATIONS

Criteria for Diagnosis of CLL

The National Cancer Institute-sponsored Working Group (NCI-WG) on CLL was charged with the task of developing guidelines for protocol studies in this disease and in that context it recommended the following three diagnostic requirements[53]:

1. An absolute lymphocytosis in the blood, with a count of $\geq 5 \times 10^9$/L, and cells morphologically mature in appearance, sustained over at least a 4-week period.
2. ≥30% lymphocytes in a normocellular or hypercellular bone marrow.
3. A monoclonal B-cell phenotype expressed by the preponderant population of blood lymphocytes with low levels of surface immunoglobulins and simultaneously showing CD5 positivity (a pan-T-cell marker).

The requirement that the lymphocytosis should be sustained over a period of time is included in these diagnostic criteria in order to exclude those conditions (such as infectious mononucleosis, pertussis, toxoplasmosis, and cytomegalovirus infection) in which lymphocytosis is transient. In our view, if a bone marrow lymphocytosis also is required for the diagnosis of CLL, it is not necessary to prove that blood lymphocytosis is of a sustained nature because none of the conditions with transient lymphocytosis are associated with bone marrow involvement.

The International Workshop on CLL (IWCLL)[54] proposed somewhat similar diagnostic criteria, but it requires $\geq 10 \times 10^9$/L lymphocytes in the blood for the diagnosis to be made if facilities to obtain phenotyping are not available. IWCLL recommended that a diagnosis of CLL can be made in a patient with $< 10 \times 10^9$/L lymphocytes in the peripheral blood, provided phenotyping is performed and reveals the pattern characteristic of CLL as described above.

The FAB Cooperative Group[55] states that when >10% cells in the blood are large (or prolymphocytes) in appearance, the diagnosis of mixed cell type CLL should be considered. In our experience, clearly identifiable forms of prolymphocytic leukemia are a consistent clinical identity. The importance of occasional prolymphocyte appearing cells in the peripheral blood of a case of CLL is controversial.

Symptoms

The usual complaints of CLL patients are weakness, easy fatigue, night sweats, fever without infections, weight loss, frequent bacterial and viral infections, increased bleeding tendencies, and exaggerated responses to mosquito or other insect bites. These are symptoms often associated with malignancy and immunodeficiency. Symptoms may be entirely absent, or only some or all may be present in varying severity.

Findings on Physical Examination

The most frequently noted abnormal finding on physical examination is lymphadenopathy. Only a single node-bearing area may be involved, or lymph nodes may be palpably enlarged in the cervical, axillary, and inguinofemoral areas. These nodes may be small (e.g., about 1 cm in diameter) or massively enlarged. The enlarged lymph nodes in CLL are almost always nontender, nonpainful, discrete, firm, and easily movable on palpation. Enlargement of spleen and liver when present may range from barely palpable to ≤15 or 20 cm below the respective costal margin. In addition, infiltration by CLL cells may be manifested in virtually all other parts of the body, including the meninges and skin.

Laboratory Evaluation

Absolute lymphocytosis in blood, with mature-appearing cells, is one of the two major presenting features of CLL (Plate 83-1). The blood lymphocyte count may range from 5 to 500 $\times 10^9$/L, but in most cases it is $> 20 \times 10^9$/L. Similarly, the differential count of bone marrow aspirate smear may reveal lymphocytes accounting for as little as 30% of all nucleated cells or as much as 99%; in the latter, the marrow is totally replaced by monotonously similar appearing lymphocytes. The overall cellularity of bone marrow is normal or increased; a hypocellular marrow is not a typical finding in CLL unless it is the result of cytotoxic therapy. Depending on the extent of lymphocytic infiltration, myeloid and erythroid precursors and megakaryocytes may be decreased or normal. Pure red cell aplasia,[56–58] however, may also occur in CLL. Bone marrow biopsy examination has become virtually routine whenever an aspiration procedure is performed in CLL. Biopsy specimens are characteristically infiltrated with lymphocytes, but the patterns of such infiltration may be diffuse, nodular, or interstitial[59–64] (Plates 83-2 to 83-4).

Lymphocytes in the blood and the marrow appear morphologically mature. However, it is now well recognized that func-

Table 83-3. Markers Useful in Distinguishing Chronic Lymphoid Leukemias

Marker	CLL	Prolymphocytic Leukemia	Hairy Cell Leukemia	Leukemic Phase of Follicular Non-Hodgkin Lymphoma	Plasma Cell Tumors	T-CLL	Sézary Syndrome
Surface immunoglobulin	Weak	Strong	Strong	Strong	Negative	Negative	Negative
Cytoplasmic immunoglobulin	−	±	±	−	++	−	−
Mouse red cell rosetting	++	−	±	±	−	−	−
Sheep red cell rosetting	−	−	−	−	−	++	++
CD2	−	−	−	−	−	++	++
CD3	−	−	−	−	−	++	++
CD4	−	−	−	−	−	+	++
CD5	++	±	−	−	−	++	++
CD7	−	−	−	−	−	++	−
CD8	−	−	−	−	−	+	−
CD19/20/24	++	++	++	++	−	−	−
Anticlass II MHC antigens	++	++	++	++	−	−	−
CD22	±	++	++	+	−	−	−
CD10	−	±	−	+	±	−	−
CD25	−	−	++	−	−	−	−
CD38	−	−	±	±	++	−	−

Symbols: +, incidence at which a marker is positive in >40% of cells in a particular leukemia; ++, 80–100%; ±, 10–40%; −, 0–9% of cases. (Modified from Bennett et al.,[55] with permission.)

tionally they are not mature inasmuch as they are arrested at an intermediate level of differentiation.[65] Lymphocytes in CLL are usually small, with the nucleus filling almost the entire cell, and the nuclear chromatin is dense and clumped and without any discernible nucleolus (Plate 83-1). Occasionally the CLL lymphocyte may be a large cell with round or somewhat notched nucleus, there may be an indistinct nucleolus, and the cytoplasm may be abundant and slightly basophilic or orthochromatic. Several morphologic variants of CLL have been described in the literature (e.g., prolymphocytic leukemia and Sézary cell leukemia).[55] Preparation of blood films may cause severe morphologic deformities of CLL lymphocytes, recognized as "smudge" cells (Plate No. 83-5).

Although CLL is characterized by leukocytosis, the proportion of neutrophils is always reduced, but to a varying degree—from as low as 1% to as high as 40%. Thus the absolute neutrophil count may be normal, extremely low, or extremely high, depending on total leukocyte count and percentage of neutrophils.

About 30% of all CLL patients may have somewhat decreased hemoglobin values or platelet counts, but these values are significantly decreased in only 15% of cases (hemoglobin <110 g/L, platelets $<100 \times 10^9$/L) at the time of initial diagnosis. Autoimmune complications, including Coombs-positive hemolytic anemia, immune thrombocytopenia, and hypogammaglobulinemia, are discussed above.

There is no characteristic abnormality of blood chemistry profile in CLL, but hypercalcemia and abnormal liver and kidney function tests may be encountered.

Differential Diagnosis

The differential diagnosis of CLL includes the entire spectrum of chronic lymphoproliferative disorders. Malignant lymphoma in leukemic phase may sometimes be indistinguishable from CLL, and the most helpful findings in such situations are phenotypic profiles of lymphocytes. Whereas the fluorescence intensity of surface immunoglobulins on B lymphocytes is bright in lymphomas, it is very faint in CLL; only B lymphocytes of CLL form rosettes with mouse erythrocytes and carry the T-cell marker denoted by CD5. T-cell CLL and morphologic variants of CLL (prolymphocytic leukemia, Sézary syndrome, hairy cell leukemia, and so forth) are distinguished by their respective characteristic phenotypic and microscopic appearances.[55] The phenotypic characteristics useful in differentiating these various disorders are summarized in Table 83-3.

Prognosis

Just as the initial extent of disease is variable in CLL, the prognosis and clinical course also are extremely variable. Some patients have a rapid downhill course and die within 2–3 years after diagnosis, whereas others have a very benign, indolent course and live for 10 or 20 years without major problems from CLL. About one-half of CLL patients have a disease course somewhere in between the two extremes. Boggs et al.[66] studied several prognostic factors and concluded that the extent of disease at initial diagnosis correlates inversely with survival.

Clinical Staging System of Rai

Building on the work of Dameshek,[4] Boggs et al.,[66] and of Hansen,[67] my colleagues and I[68] were able to devise a clinical staging system in which patients with minimum evidence of disease (those merely satisfying the minimum diagnostic criteria) were considered to be in the earliest stage of disease, whereas those demonstrating significant compromise of bone marrow function (as an index of high leukemic cell burden) were considered to be in advanced stages. This staging system is detailed in Table 83-4.

Table 83-4. Rai Clinical Staging Systems

Level of Risk	Stage	Description
Low[a]	0	Lymphocytosis only (in blood and marrow)
	I	Lymphocytosis plus enlarged nodes
Intermediate[a]	II	Lymphocytosis plus enlarged spleen and/or liver with or without enlargement of nodes
	III	Lymphocytosis plus anemia (hemoglobin <110 g/L) with or without enlarged nodes, spleen, liver
High[a]	IV	Lymphocytosis plus thrombocytopenia (platelets $<100 \times 10^9$/L) with or without anemia and/or enlarged nodes, spleen, liver

[a] Modified Rai system.

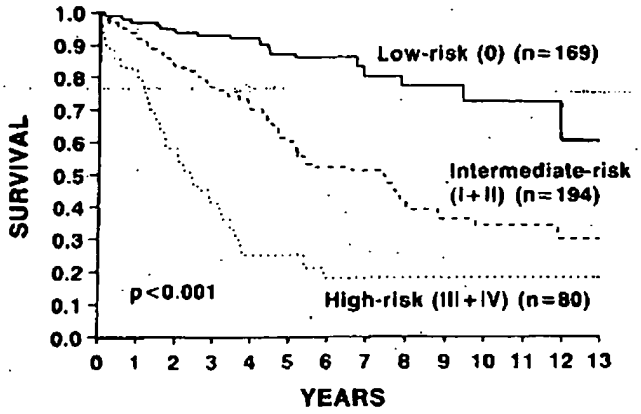

Fig. 83-1. Survival according to the modified Rai staging criteria in 443 patients with CLL followed at the Postgraduate School of Hematology, Barcelona, Spain. (From Montserrat and Rozman,[70] with permission.)

The definition of lymphocytosis in our original description consisted of a 15×10^9/L or higher absolute lymphocyte count in blood and ≥40% of lymphocytes in the marrow differential count of all nucleated cells. However, following the recommendations of the NCI-WG[53] and the IWCLL[54] detailed above, we suggest that these thresholds be modified to $\geq 5 \times 10^9$/L and >30% in blood and marrow, respectively. The presence of palpably enlarged lymph nodes was found to be of prognostic value (stage I), but it did not seem to make much difference whether adenopathy was bulky or minimal or whether it involved a single node-bearing region or was generalized. Median survival correlated inversely with the clinical stage: stage 0, 12+ years; stage I, 8.5 years; stage II, 6 years; stage III, 1.5 years; and stage IV, 1.5 years.[68]

Modified Rai System

Although several investigators confirmed the validity of the staging system of Rai et al. as a reliable predictor of survival time in CLL, many workers found that having as many as five stages in the system made it difficult to plan prospective therapeutic trials. We had, however, acknowledged in our original proposal[68] in 1975 that in the actuarial survival curves, there were indeed only three, not five, distinct patterns: (1) stage 0, (2) stages I and II combined, and (3) stages III and IV combined.[68] Therefore, a formal modification[69] of the staging system was published in 1987, which assigns stage 0 to the low-risk group, stages I and II combined to the intermediate-risk group, and stages III and IV combined to the high-risk group (Table 83-4). The survival curves according to the modified Rai system in a large series of CLL patients followed in the Hematology Clinic of the University of Barcelona in Spain[70] are shown in Figure 83-1. These curves are statistically significantly different from each other and demonstrate that the low-risk group patients have the best outlook for survival. The NCI-WG recommends[53] using the modified Rai staging system for prospective therapeutic trials.

Staging System of Binet and Colleagues

The only other staging system that has found wide acceptance in clinical practice is the one devised by Binet et al.[71] This system is similar to the Rai system in concept. Stage C consists of all patients who have anemia (hemoglobin <100 g/L) and/or thrombocytopenia (platelets $<100 \times 10^9$/L). All other (non-C) patients are divided into A or B stages depending on the number of lymphoid-bearing areas palpably enlarged, two or less for A and more than three for B (there are five of these areas: the cervical, axillary, and inguinofemoral nodes; spleen; and liver). This three-stage system is an excellent predictor of survival and is useful in planning therapeutic trials. Binet et al. have observed that the survival times of stage A patients do not differ from those of age- and sex-matched normal members of the French population. Inasmuch as stage A patients include those who have splenomegaly with one area of lymphadenopathy (or two areas of adenopathy without splenomegaly), it is somewhat surprising that the life expectancy of such patients is equal to that of the nonleukemic normal French population. Conceptually, in our opinion, the stage 0 subgroup of Binet's A should have a better prognosis than all other (non-stage-0) patients in stage A. In actual practice, both the Rai and the Binet systems are used in clinical management and in therapeutic protocols.

Other Staging Systems

The IWCLL recommends the use of an integrated Binet and Rai system in which each Binet stage (A, B, or C) is subclassified according to the corresponding Rai stage.[54] However, clinicians use either of the two systems without the recommended integration.

Jaksic and Vitale's[72] total tumor mass estimation yields a score indicating the size of the spleen and the largest palpable lymph node, while also including a factor related to the blood lymphocyte count. Other systems proposed include those of Mandelli et al.,[73] Lee et al.,[74] Baccarini et al.,[75] Skinnider et al.,[76] and Paolino et al.[77] Each of these systems has certain advantages and helps a physician in individual cases but none has found wide usage, perhaps because the Rai and Binet methods are simple to apply and succeed in segregating large populations of CLL patients into distinct groups of survival outlook.

Additional Prognostic Features

Numerous clinical, hematologic, and laboratory abnormalities, as well as immunophenotypic and cytogenetic characteristics have been reported to be indicative of adverse prognosis in CLL. Some of these are listed in Table 83-5. Perhaps only a

Table 83-5. Factors Associated with a Poor Prognosis

Clinical
Lymphadenopathy
Splenomegaly
Hepatomegaly
"Bulky" disease
Poor performance status
Hematologic
Anemia
Thrombocytopenia
Large and atypical lymphocytes in blood
Diffuse bone marrow histopathologic pattern
Laboratory abnormalities
Increased serum lactate dehydrogenase level
Hypoalbuminemia
Increased serum calcium level
Cytogenetic abnormalities
Complex and multiple cytogenetic abnormalities (e.g., 14q32, 12+, others)
Immunologic
Hypogammaglobulinemia
Immunophenotype (different abnormalities related to poor prognosis (e.g., $SmIg^{+++}$, $CD5^-$, $CD23^-$))
Increased serum-soluble CD25 receptors
Increased serum-soluble CD23 receptors
Kinetic parameters
Rapid doubling time
Others
Poor response to therapy

(Adapted from Montserrat and Rozman,[70] with permission.)

few of these features have a consistent impact on prognosis and are described below.

Lymphocyte Doubling Time

Although the lymphocyte count acts as a continuous variable as a prognosis indicator, we have not found a threshold (such as a cut-off level of 40–50 × 10^9/L) count consistently reliable. However, the rate of increase of the absolute lymphocyte count in the blood of CLL patients not receiving cytotoxic therapy has proven to be a reliable indicator of disease activity. A serial plotting of blood lymphocyte counts provides a measure of this activity and either by extrapolation or by actual observation it can be determined whether the blood lymphocyte count doubles slowly (≥12 months) or rapidly (<12 months); the latter is associated with a worse prognosis.[78–80]

Bone marrow histopathology: The pattern of lymphocytic infiltration in the bone marrow biopsy specimens (Plates 83-2 to 83-4) can be classified as either diffuse or nondiffuse. The nondiffuse pattern may be nodular, interstitial, or mixed nodular and interstitial. Patients with diffuse infiltration have a worse prognosis than those with nondiffuse. The diffuse pattern is seen most frequently in advanced clinical stage CLL, while a nondiffuse infiltration is the more likely pattern in early stages of CLL.[59,62,63,81]

Immunophenotypic Features

A large, prospective study of flow-cytometric immunophenotyping in Denmark provides the most systematic analysis of prognostic value of these findings.[82] There were 503 $CD5^+$ and 37 $CD5^-$ cases. The survival of $CD5^-$ patients was on the borderline of being significantly shorter than that of $CD5^+$ patients. Most $CD5^-$ patients had malignant lymphocytes with strong sIgM fluorescence, and were $FMC7^+$ and $CD23^-$, indicating that $CD5^-$ cases represent an atypical variant of CLL.[82] Among the $CD5^+$ cases, by Cox multiple regression analysis a few features emerged with independent prognostic importance: higher age, low CD23 expression and high sIgM fluorescence intensity, and advanced clinical stage were all associated with worse prognosis. CD20, CD21, and CD22 expression did not have prognostic importance.[82]

Cytogenetics

A clonal chromosomal abnormality indicates a poorer prognosis as compared with a normal karyotype.[24] Although trisomy 12 as a single chromosomal abnormality is an adverse prognostic sign in CLL, multiple chromosomal abnormalities are associated with a worse prognosis. Patients with 13q abnormalities do not have as bad a prognosis as those with 12+ or 14q+.[24]

Prediction of Clinical Course in Patients with Early Stages

Neither the Rai nor the Binet system of clinical staging can reliably predict the clinical course of patients in the nonadvanced stages (0, I, and II for the Rai and A and B for the Binet) of CLL. It is widely recognized that there is a group of patients in these stages whose disease course remains indolent for prolonged periods (several years) and another group with a relatively progressive and active course. Several prognostic factors have been tested by numerous investigators since about the 1970s. These include a blood lymphocyte count above or below a certain threshold[75,83,84]; lymphocyte morphology and size[79,85]; serum levels of several enzymes (e.g., lactate dehydrogenase[86] and deoxythymidine kinase[87]); serum β-microglobulin levels[88]; phenotype of blood lymphocytes[82,89]; and chromosomal abnormalities.[24] The value of these criteria for predicting the clinical course of CLL has not been universally accepted.

Table 83-6. Definition of Smoldering CLL

Patients in Binet's stage A
Nondiffuse lymphocytic infiltration in bone marrow biopsy
Lymphocyte doubling time >12 months
Blood lymphocyte count ≤30 × 10^9/L
Hemoglobin ≥13 g/dl

(From Montserrat and Rozman,[70] with permission.)

Patients with early stages of CLL whose blood lymphocyte doubling time is long (>12 months) and whose bone marrow biopsy shows a nondiffuse pattern of lymphocytic infiltration tend to have an indolent course of the disease. The Spanish group[90] retrospectively tested certain criteria associated with nonprogressive (or stable) CLL among patients in Binet's A stage, which they call "smoldering CLL." They noted that (in addition to the two criteria mentioned above), a relatively low (≤30 × 10^9/L) absolute lymphocyte count and a relatively high (≥13 g/dL) hemoglobin (Table 83-6) characterize the category of smoldering CLL. The patients with smoldering CLL were found to have life expectancies no different than those of an age- and sex-matched control population and a significantly lower risk of disease progression compared with the other Binet's stage A patients not meeting the criteria of smoldering CLL (and were deemed to have "active" CLL)[70] (Table 83-7). The French Cooperative Group[91] on CLL recommended similar criteria associated with smoldering CLL in early stages of the disease. Thus, it seems that we are approaching a successful resolution of the problem of predicting disease activity in the nonadvanced stages of CLL.

CLL in the Younger Age Group

CLL is a disease of the elderly; the median age at diagnosis is 55 years. However, with the easy availability of routine blood counts in today's society, CLL is being diagnosed in increasing frequency both at earlier stages of the disease as well as in younger age groups. About 12% of patients are <50 years of age at diagnosis. Although nearly two-thirds of younger age CLL patients are >40 years of age, a recent Mayo Clinic report shows 18 years as the lower end of the age range.[80] With the advent of newer and more aggressive therapies in CLL, several studies have been conducted to determine whether younger age CLL patients have different outcomes than those in the more typical, older age groups. These studies show that there are no differences in the presenting features, treatment response rates, and median durations of response between the younger and the older age groups.[92–96] The Mayo Clinic study was confined to the prognostic features of nonadvanced stages of CLL in the younger patients and it showed that the survival curves for stages 0 and I were virtually superimposable with a median duration of 140 months and was significantly longer

Table 83-7. Disease Activity in Smoldering Versus Active CLL

	Smoldering CLL[a] (%)	"Active" CLL (Nonsmoldering)[b] (%)	
Risk of progression			
at 3 yr	8	57	
95% CI	6–16	42–72	
at 5 yr	13	57	
95% CI	3–24	42–72	$P < 0.001$
Surviving at 10 yr	78	43	
95% CI	56–99	22–64	$P < 0.05$

Abbreviation: CI, confidence interval.

[a] 38 patients.

[b] 161 patients.

(Data from Montserrat and Rozman.[70])

than the median duration of 60 months for stage II patients. On multivariate analysis of several factors, only clinical stage (0 and I versus II) and lymphocyte doubling time (>12 versus ≤12 months) emerged as prognostically useful among younger age CLL patients in the nonadvanced stages.[80] These observations are helpful in planning long-term therapeutic options for these patients who do not find it particularly reassuring to be informed when they are 40 years of age that their median life expectancy is 10 or 12 years. The same prognosis may not have a grim impact on a 70-year-old person. Therefore, although it is helpful to know that there are no unique prognostic factors for the younger CLL patients, the ability of the physician as well as the patient to weigh the risks and benefits of various treatment options is greatly influenced by the patient's age.

THERAPY

Period of Observation Without Cytotoxic Therapy

It is prudent to withhold cytotoxic therapy after the initial diagnosis of CLL has been established.[97] A vast majority of patients can withstand a period without such therapy. As the clinical course is highly variable in this disease, the physician can use the therapy-free few weeks to observe whether the disease is stable or progressive in an individual patient on the basis of the following criteria:

1. The rate of increase of blood lymphocyte count is charted to determine whether the projected doubling time is long (>12 months) or short (≤12 months).
2. The clinical stage is clearly established.
3. The pattern of lymphocytic infiltration in bone marrow biopsy specimen is noted.
4. The presence or absence of constitutional symptoms is noted.

The therapy-free period may be extended indefinitely for those patients whose disease appears to be indolent; such patients may need to return to the clinic less frequently (e.g., at 3-month intervals). A minority of patients have an aggressive course of CLL and require institution of some therapy within 2–4 weeks from the time of initial diagnosis.

Indications for Therapeutic Intervention

A decision to start antileukemia therapy is made in the presence of any of the following indications[97]:

1. Disease-related progressive symptoms (e.g., weight loss without trying, fever without overt infection, night sweats, weakness, or easy fatigability).
2. Progressively worsening anemia or thrombocytopenia.
3. Autoimmune (Coombs-positive) hemolytic anemia or autoimmune thrombocytopenic purpura.
4. "Bulky" lymphadenopathy that is getting progressively worse poses risk to the patient from pressure on underlying tissues, or causes significant cosmetic problems.
5. Massive splenomegaly that is worsening progressively or results in hypersplenism.
6. Progressive hyperlymphocytosis. It is not possible to set a rigid upper threshold for the blood lymphocyte count that must be met before starting therapy, but it is our current practice not to allow this count to be $>150 \times 10^9$/L. Hyperviscosity syndrome associated with hyperlymphocytosis in CLL can be catastrophic.[98,99] As noted earlier, the rate of increase of blood lymphocyte count is of equal importance; thus a short doubling time (≤12 months, actual or by extrapolation) is an indication for therapeutic intervention.
7. Increased susceptibility to bacterial infections.[50,51] This may result from marked hypogammaglobulinemia, in which case intravenous high-dose γ-globulin therapy has a proven protective effect.[100] Severe neutropenia or agranulocytosis may occur in CLL, and may play a major role in the development of bacterial sepsis.[51]

Choices of Therapeutic Modalities

Alkylating Agents

Chlorambucil and cyclosphosphamide are the most frequently used initial drugs of choice. Chlorambucil is administered orally and is readily absorbed from the gastrointestinal tract. There are two methods of treatment with this drug: small-dose continuous therapy (0.07 mg/kg/day with adjustments as needed by monitoring blood counts at weekly or biweekly intervals) or large-dose bolus intermittent therapy (0.7 mg/kg at intervals of 3 or 4 weeks).[101] Both methods are approximately equally effective, markedly reducing the size of previously enlarged lymph nodes or spleen, but patient compliance is perhaps better with intermittent therapy. The premise[102,103] that the intermittent method may enable recovery of normal hematopoietic elements in the marrow before regrowth of leukemic cells between two successive doses and thus be superior to daily continuous administration of chlorambucil was not conclusively proven to be the case in a randomized trial sponsored by the Cancer and Leukemia Group B (CALGB).[101] Our own preference is for intermittent chlorambucil, but both methods are commonly used by physicians caring for CLL patients. The dose-limiting toxicity is bone marrow suppression, which is reversible if it is recognized promptly by frequent blood counts. Nausea, vomiting, mucositis, and so forth, are not major problems with chlorambucil.

The second most frequently used alkylating agent in CLL is cyclophosphamide, which may be given orally or intravenously. This drug, like chlorambucil, is also given either on a daily basis (50–150 mg/day PO) or intermittently (1,000–1,200 mg every 2–4 weeks PO). The intravenous dose is the same as the intermittent oral dose. Cyclophosphamide is equal to chlorambucil in its effectiveness in control of CLL, but some patients who are starting to show refractoriness to the latter respond to the former. Besides bone marrow suppression, chemical cystitis is a major side effect of cyclophosphamide, but is avoidable in most cases by ensuring adequate hydration and advising the patients to urinate frequently after each intermittently administered dose. The incidence of nausea is somewhat greater than with chlorambucil. Both drugs are effective as first-line single-agent therapy in CLL. Other alkylating agents (e.g., melphalan or nitrogen mustard) may also be effective, but they are associated with considerable toxicities and are rarely used in CLL.

Fludarabine monophosphate is a fluorinated analogue of adenine that is resistant to deamination by the enzyme adenosine deaminase. This drug has proved an effective therapy for CLL patients who are resistant to alkylating agents.[104] Fludarabine is given intravenously at a dose of 25 mg/m^2/day for 5 consecutive days every month. Usually four to six treatments at monthly intervals are required to achieve the maximally achievable benefits from this drug. Although fludarabine does not cause nausea, vomiting, and hair loss, prolonged use may result in cumulative myelotoxicity and infectious complications. Precautions against tumor lysis syndrome during the initial phase of therapy with fludarabine are advised. Addition of prednisone does not increase response rates of fludarabine, but may increase the risk of opportunistic infections.[105,106]

Other Chemotherapeutic Drugs

Alkylating agents and fludarabine are the only cytotoxic drugs used as single agents in CLL, but vincristine, doxorubicin, nitrosoureas, and others are administered as part of several

combination chemotherapy protocols. Even though vincristine has been administered frequently in CLL (being a part of several treatment schedules tested in lymphomas and used when a CLL patient is refractory to single-agent therapy), there is no clear evidence that this drug is indeed useful in the treatment of CLL. Considering the potential of significant peripheral neuropathy associated with vincristine therapy in CLL patients, who usually are elderly, we recommend either reduced dosage or avoidance of this drug when using a combination chemotherapy schedule that includes it.

Glucocorticosteroids

Glucocorticosteroids (e.g., prednisone) are frequently used in CLL either as single agents in the management of autoimmune hemolytic anemia and thrombocytopenia or as part of combination chemotherapy protocols in other cases. Prednisone has significant lymphocytolytic effect; it causes marked reduction in previously enlarged nodes and spleen, and after an initial phase of causing a further increase in blood lymphocytosis, eventually results in significant decrease when therapy is continued for several days or a few weeks. Side effects of prednisone use (especially in elderly CLL patients) that should be kept in mind are increased blood sugar levels, worsening of pre-existing osteoporosis, psychiatric reactions ranging from euphoria to severe depression, and increased susceptibility to infections, particularly reactivation of old healed tuberculous lesions. Prednisone is given orally on an intermittent schedule of 40–80 mg/day for 5–7 days every month. In some patients with persistent chronic hemolysis, a lower dosage maintenance schedule (e.g., 5–15 mg/day or twice a week) may be necessary.

Androgens or Anabolic Steroids

Androgens or anabolic steroids[107] have been used in CLL patients with marked anemia (considered to be due to leukemic infiltration of bone marrow or erythroid hypoplasia) to stimulate erythropoiesis. These agents are not uniformly effective but in some patients have been very beneficial; therefore, in selected situations, their use is justifiable. Side effects include hepatic toxicity, hirsutism, and prostatism. There have been reports of diethylstilbestrol causing significant reduction in blood lymphocyte counts when used in those patients with prostate cancer who happened also to have CLL,[108] but these results have not been confirmed in any controlled trials in CLL.

Radiotherapy

The most frequently used form of radiotherapy is splenic irradiation,[109,110] which in selected patients results in prompt reduction in the size of an enlarged spleen, accompanied by evidence of partial control of overall disease. This treatment is particularly beneficial in patients with marked splenomegaly who are not responsive to chemotherapy. Irradiation of large, bulky lymphoid masses localized in one region, nonresponsive to chemotherapy, is also an effective method of treatment. Experimental therapies, including extracorporeal irradiation of blood,[111] mediastinal irradiation,[112–114] total body irradiation,[109,115,116] and administration of radioactive isotopes,[117] have proved to be either too toxic or ineffective. A consultation with a radiation oncologist experienced in treating CLL and other hematologic malignancies is recommended in deciding whether radiotherapy is advisable.

Splenectomy

Splenectomy[118–123] is an effective treatment for CLL in specific clinical situations. Patients with extensive splenomegaly unresponsive to chemotherapy and with significant anemia or thrombocytopenia attributed to hypersplenism (some of these patients are not considered for splenic irradiation because of fear of worsening anemia or thrombocytopenia, and some of them may have failed radiotherapy) and patients whose response to steroid therapy is inadequate in the presence of autoimmune anemia are among those considered to be candidates for splenectomy. Beneficial response to splenectomy may last from a few months to several years. Pneumococcal vaccine should be administered before surgery.

Leukapheresis

Leukapheresis[124,125] has a very limited role in the long-term management of CLL. When the blood leukocyte count is $>500 \times 10^9$/L either at diagnosis or during the course of the disease, catastrophic complications of hyperviscosity and thromboembolic phenomena may be avoided by resorting to intensive leukapheresis (using one of the automatic cell-separating machines) while simultaneously providing adequate hydration and initiating cytotoxic chemotherapy. Leukapheresis as the sole therapeutic measure is of no benefit because the reduction in blood lymphocyte count so achieved is very transient.

Criteria for Evaluating Response

The NCI-WG[53] and the IWCLL[54] have separately proposed a series of criteria to objectively assess complete or partial remission in CLL. To qualify for complete remission a patient must have no adenopathy, no hepatosplenomegaly, no constitutional symptoms, normal hemogram (hemoglobin >110 g/L, platelets $>100 \times 10^9$/L, absolute lymphocytes $<4 \times 10^9$/L, and absolute neutrophils $>1.5 \times 10^9$/L), and the bone marrow must contain <30% lymphocytes. To qualify for partial remission, IWCLL criteria require an improvement in clinical stage (e.g., from Binet's C to B or A or from Binet's B to A). The NCI-WG recommends a similar approach (i.e., improvement in clinical stage), but in addition defines partial remission by a >50% reduction in the previously enlarged nodes, spleen, or liver, together with a >50% improvement of peripheral blood values over baseline (when they do not approach the levels described for complete remission).

Therapy Based on Clinical Stage

Low-risk (stage 0) patients should not be started on cytotoxic therapy unless significant constitutional symptoms develop or there is evidence of an active clinical course as described above.

Intermediate-risk (stage I and II) patients should also be observed without antileukemia therapy until there is evidence of an active clinical course as described above or of any of the indications for therapeutic intervention also as enumerated above. In randomized studies CALGB[126] and the French Cooperative Group[127] assigned early-stage patients to chlorambucil therapy or observation alone. Both these studies showed that early treatment with chlorambucil did not improve survival, although it did correlate with a somewhat slower progression to the more advanced stages. If treatment is indicated, chlorambucil as a single agent is the appropriate first-line therapy. The therapeutic end point is unclear; we do not know whether pushing treatment to try to achieve a complete remission is necessarily beneficial for the patient. The current practice, however, is to at least try to eliminate whatever indication required initiation of therapy in the first instance, and if possible, to maximize the best achievable level of response without subjecting the patient to undue toxicity.

High-risk (stages III and IV) patients have a uniformly poor

APPROACH TO THERAPY FOR CLL

We use the following diagnostic criteria for CLL: an absolute lymphocytosis in blood with mature-appearing lymphocytes; lymphocytosis in the marrow, which is hypercellular or at least normocellular; and a monoclonal B-cell phenotype of the preponderant population of blood lymphocytes with low levels of surface immunoglobulins and simultaneously expressing CD5 positivity. The threshold for the absolute lymphocyte count in the blood is 5×10^9/L and the threshold for the proportion of lymphocytes among all nucleated cells in the marrow is 30%.

We begin treatment if a patient in the low- or intermediate-risk category shows a rapid rate of increase in blood lymphocyte count (doubling time, actual or projected, of ≤12 months) on several weeks of observation without cytotoxic therapy and, in addition, the marrow biopsy shows diffuse lymphocytic infiltration. We initiate therapy in all high-risk category patients. More specifically, we use the seven indications listed in the text; if any of these is present, we initiate therapy.

If, for any reason, the patient is not going to be placed on a research protocol for front-line therapy, in routine clinical management we continue to use chlorambucil for low- and intermediate-risk (stages 0, I, or II) CLL. We recommend an intermittent schedule in which 40 mg/m² total dose, taken orally in 1 day or divided over 2 days, is repeated every 4 weeks. In high-risk (stages III or IV) patients, we add prednisone (60 mg/day PO) for 5 days at each 4-week chlorambucil therapy. All patients starting this therapy for the first time also take allopurinol (300 mg/day PO) for 1 week with each cycle of therapy with chlorambucil. If there is no evidence of hyperuricemia, we do not continue with allopurinol after 2–3 months. When prednisone is prescribed, we monitor blood sugar levels to ensure that complications from hyperglycemia do not occur.

All patients have a weekly monitoring of blood counts and serum chemistries for the first 8 weeks. By then, each patient's nadir counts and rate of recovery are well established, as is also the dosage of chlorambucil (which needs to be adjusted upward or downward depending on the response in blood counts). Thereafter, patients return for an examination every 3–4 weeks.

As soon as we recognize that chlorambucil is not optimally effective we switch to the second-line drugs. Fludarabine is our drug of choice for all patients who show inadequate degree of response to chlorambucil or who start to show evidence of recurrent disease after having had a response to initial chlorambucil. Fludarabine dosage is 25 mg/m²/day IV for 5 days each month. We do not use prednisone with fludarabine because with this combination a patient has an increased risk of developing opportunistic infections. A maximally achievable beneficial response is usually attained after four to six cycles of fludarabine, when this therapy is stopped.

Patients who do not respond to fludarabine or relapse after an initial response are offered 2-chlorodeoxyadenosine (2cdA). 2cdA offers some hope for response, particularly in patients who have previously not been heavily treated with fludarabine and other drugs. In our experience combination chemotherapy schedules such as COP, CHOP, M-2 are not very effective.

For CLL patients ≤50 years of age, we consult with the bone marrow transplantation service. Allogeneic marrow transplantation is recommended if an HLA-matched sibling donor is available; otherwise autologous marrow transplantation is considered.

We use prednisone for patients with autoimmune anemia/thrombocytopenia. If prednisone does not produce satisfactory results, we add a 2–3-month trial of intravenous γ-globulin therapy, which consists of 200 mg/kg/day over a 3-hour period for 5 days initially (loading dose), followed by only one dose every 3 weeks. If there is a satisfactory response, we continue with prednisone plus intravenous γ-globulin therapy for 6–12 months. If the results are inadequate, we consider splenectomy.

With respect to pure red cell aplasia in CLL, if there is a true reticulocytopenia with severe anemia in Coombs-negative patients or if the marrow shows markedly decreased or absent erythroid precursors, we recommend Cyclosporin A at a dose of 600–1,000 mg/day PO; tapered to 400–600 mg/day after 2 weeks. Close monitoring of serum BUN, creatinine, and liver enzymes is necessary to adjust the dosage and to prevent undue toxicity. If a beneficial response is likely to occur, it will become evident within 2 weeks, by an increase in either reticulocytes or hemoglobin.

The patients to whom we give intravenous γ-globulin are primarily CLL patients who have had pneumonia or any other major documented bacterial infection, especially if their baseline serum IgG level is <6.0 g/L. We start intravenous γ-globulin without waiting for a second episode of infection. We give 200 mg/kg over a 3-hour period every 3 weeks for 6 months. Following that, we reduce the frequency of infusion to every 6 or 8 weeks (especially if the initial therapy has protected the patient from recurrence of bacterial infections).

We treat infections in CLL very vigorously. We obtain multiple blood, urine, and sputum cultures if indicated, and consult with colleagues in infectious diseases for their recommendation of empirically selected antibiotics. We discontinue or taper prednisone therapy. We actively look for tuberculosis and for infections caused by *Pneumocystis carinii* and other opportunistic organisms.

By way of supportive care we give packed cell transfusions to all anemic patients who are symptomatic from anemia and have not responded to prednisone. If the hematocrit is ≤24%, we tend to give transfusions to maintain it at >28%. If a patient's base-line serum erythropoietin level is low or within normal range we initiate a 4-week therapeutic trial with recombinant erythropoietin injections subcutaneously. This therapy is continued only if the initial trial reveals some improvement in anemia. We do not use prophylactic oral antibiotics, and we do not have a set policy concerning the use of pneumococcal vaccine or influenza vaccine.

We are very sensitive to patients' psychosocial needs and to their ability to cope with the stress of a terminal disease. We enlist the help of a psychiatrist specializing in oncology and of a social worker who has experience in oncology. We spend a considerable amount of our own time talking to the patients and their families—reassuring them, answering their questions, and helping them to anticipate problems rather than to have the problems descend on them without adequate preparation or warning.

prognosis and should be started on cytotoxic therapy. A large trial by CALGB[101] revealed that if at least a partial (complete if possible) remission is achieved after chlorambucil and prednisone therapy, there is a significant improvement in survival as compared with survival in those patients who failed to achieve such a response. This was the first study that defined the therapeutic end point in advanced CLL (i.e., a partial or complete remission). The French Cooperative Group[128] demonstrated a significantly better survival of advanced stage patients after treatment with COP (cyclosphosphamide, vincristine [Oncovin], prednisone) together with low-dose doxorubicin (CHOP) as compared with patients who received COP without doxorubicin.[128] The results of this study, however, have not been confirmed by subsequent trials, nor has there been any evidence that a somewhat higher response rate with CHOP provides a survival advantage over COP, or chlorambucil and prednisone therapy.[129–133]

Second-Line Therapy

Fludarabine

Fludarabine has proven to be an extremely effective drug for CLL patients who have failed prior therapy with an alkylating agent.[104–106] A large proportion of such patients obtain objective responses and responding patients have an improved survival.

Combination Chemotherapy

Other multiagent combination chemotherapies (e.g., POACH, [COP plus cytosine arabinoside and doxorubicin],[134] devised at the M.D. Anderson Hospital in Houston, Texas) or the M-2 protocol[135] (COP plus melphalan and carmustine, developed for treatment of multiple myeloma at the Memorial Sloan-Kettering Cancer Center in New York) also have some promise in the management of advanced stages of CLL but the results are not uniformly consistent.

Special Therapeutic Issues

High-Dose Intravenous Immunoglobulin

As mentioned earlier, significant hypogammaglobulinemia is frequently observed in CLL, rendering these patients highly vulnerable to bacterial infections. With the availability of purified immunoglobulins for intravenous administration, it is now possible to treat those CLL patients who are at increased risk of infections. In a multi-institutional, placebo-controlled randomized trial,[100] it was observed that replacement therapy with intravenous γ-globulin provides a significant protection from major bacterial infections in CLL patients who would otherwise be at risk. The γ-globulin dose in this study was 400 mg/kg body weight every 3 weeks for 1 year. Although such therapy appears rather expensive, when one considers the potential costs of treating bacterial pneumonia in a hospital inpatient setting, the cost of such preventive measures becomes justifiable in certain selected patients who are at high risk of infections. We have been using a lower dosage (200 mg/kg body weight) of intravenous immunoglobulin, which appears to provide protection from infections at significantly reduced costs. A multicenter trial comparing a high dose (500 mg/kg/mo) with a lower dose (250 mg/kg/mo) has been conducted and the preliminary results show that both dosages are equally effective in preventing bacterial infections in the at-risk patients with CLL.[136] Thus, if the patients are selected for their previous history of major bacterial infections and/or severe hypogammaglobulinemia and the dosage of intravenous immunoglobulin is lowered, the overall cost of such a preventive measure would not remain as big an issue as was previously considered.[137] This therapy is well tolerated and can be given at the patient's home or in outpatient clinics.

Interferon-α

Although initial results[138] with IFN-α in advanced stages of CLL were very disappointing, subsequently performed studies suggest that this agent may benefit patients in early stages of the disease, or when the tumor burden is relatively low.[139–142] The clinical benefits from a combination of IFN-α and chlorambucil and prednisone are being studied.[143] The mechanism of action of IFN-α in CLL is not understood. In vitro, it causes differentiation of CLL cells,[144] and there are also data to suggest that it inhibits apoptosis of CLL cells. However, there is no evidence of direct cytotoxicity from IFN-α; it may interfere with cellular interactions necessary for the survival and growth of CLL cells, or alternatively, it may inhibit the proliferation of the small fraction of clonogenic CLL progenitors.[145] Until more data from some of the recently completed trials become available, IFN-α should be considered an experimental agent in the treatment of CLL.

Pure Red Cell Aplasia

Pure red cell aplasia is a relatively rare cause of anemia in CLL. Some studies suggest that suppressor cytotoxic T cells exert inhibitory effects on erythroid progenitor cells in the bone marrow.[56–58] Therefore, an immunosuppressive agent such as Cyclosporin A has been proposed for therapy of pure red cell aplasia with the objective of attacking the erythropoiesis-inhibiting effect of suppressor T cells.[56] Since the first report of Chikkappa et al.[146] of successful treatment of pure red cell aplasia in CLL with Cyclosporin A, others[147,148] have confirmed these results in a small number of patients. This therapy is well tolerated, the only reported side effect being mild and reversible renal toxicity. A reticulocyte response is noted within 2 weeks of therapy and is soon followed by an increase in hemoglobin levels. We have observed[147] that not only hemoglobin but also platelet counts increased in a CLL patient who had significant, refractory anemia and thrombocytopenia before Cyclosporin A therapy.

Late Complications and Terminal Events

The quality of life and performance status of CLL patients gradually deteriorate with progression of the disease, and a refractoriness to all cytotoxic therapies becomes increasingly evident. Marked degree of persistent anemia is not unusual in the last phases of this disease and the patients require frequent and regular transfusions of packed red cells. Recombinant human erythropoietin may be effective in some patients with anemia. Although platelet transfusions are not routinely recommended in patients with profound degrees of thrombocytopenia who have no evidence of any bleeding, such transfusions are necessary in the presence of bleeding.

Infections

Infections from bacterial, viral, and fungal agents are the most important cause of morbidity and mortality in CLL.[50,51] There are several factors that contribute to the increased incidence of infections in CLL, but advanced stages or long duration of the disease, hypogammaglobulinemia and neutropenia (from CLL or from myelosuppression effects of chemotherapy) are the most significant. The new nucleoside analogues, which have found increasing usage in the therapy of CLL, are known to cause marked lymphopenia, especially of the T-helper-cell populations.[104–106] This in turn renders the patients vulnerable

to infections with opportunistic organisms. Therapy is directed at identification of the causative organisms and use of the appropriate antibiotics after consultation with an infectious diseases specialist. Judicious use of granulocyte-stimulating factor is helpful in septic patients with chemotherapy-induced neutropenia. Prophylactic use of high-dose intravenous immunoglobulin has been discussed above. Prophylactic use of antibiotics in neutropenic or hypogammaglobulinemic patients is not recommended.

Richter Syndrome

About 1–10% of patients with CLL develop a large cell lymphoma. It is known as Richter syndrome because it was Richter who first described this association.[149] In a retrospective review of 1,374 CLL patients seen at the M.D. Anderson Cancer Center during a 20-year period between 1972 and 1992, 2.8% were reported to have developed Richter transformation.[150] Richter syndrome is often characterized by sudden clinical deterioration, development of systemic symptoms, and usually a rapid increase in the size of a lymphoid mass at one site. Less frequently, a monoclonal gammopathy or lytic bone lesions are observed. The histology of lymphoma is either of diffuse large cell type or its immunoblastic variant.[150] One of our patients developed a lymphoma of Burkitt type pathology. Immunoglobulin gene rearrangements and light chain isotype analyses in the study at the M.D. Anderson Cancer Center suggest that CLL and Richter syndrome had a common origin, indicating that a bona fide transformation occurred in the CLL cells.[150] The published literature, however, supports both theories[151,152]: some studies show that lymphoma cells and CLL cells have identical features, while other studies show that lymphoma cells arise de novo with characteristics distinct from those of CLL cells. There have been separate reports of two cases extensively studied with molecular markers, one conclusively proving a common origin and another equally conclusively revealing that CLL cells and lymphoma cells were clonally distinct.[151,152] These reports prove that diffuse large cell lymphoma may occur both ways: a transformation of the original clone of CLL cells as well as a development of a new or second malignancy. It is customary to treat patients with Richter transformation with chemotherapeutic agents known to be effective in treating de novo diffuse large cell lymphoma. Therapy has so far proven to be uniformly unsuccessful and the overall survival of patients is approximately 6 months after Richter transformation.

Prolymphocytoid Transformation

In addition to Richter transformation, prolymphocytoid transformation may occur terminally in about 10% of cases with CLL. The morphology of blood lymphocytes changes into that of a large cell with convoluted nucleus, immature-appearing nuclear chromatin, and one or two large nucleoli. Therapy of this complication also is unsatisfactory. Besides the drugs commonly used in therapy of CLL, antilymphoma agents have also been tried but the success rate has been low. Acute leukemia and multiple myeloma are extremely rare late events in CLL.

CLL patients are known to have a higher incidence of developing a second malignancy (such as cancer of the gastrointestinal tract, lung, or any other organ) than the general population. Patients with CLL also have a high risk of developing skin cancers.

Paraneoplastic Pemphigus—An Autoimmune Complication

Anhalt et al.[153] have suggested that the term paraneoplastic pemphigus, which is clinically distinct from pemphigus vulgaris and pemphigus foliaceus, be applied to the painful, persistent, and treatment-resistant erosions of the oral mucosa, vermilion borders of the lips, and conjunctivitis that appear in patients with various types of cancers, including CLL. These acantholytic mucocutaneous lesions are characterized by autoantibodies that are pathogenic after passive transfer.

FUTURE DIRECTIONS

Although chlorambucil and cyclosphosphamide have been the mainstays of chemotherapy in CLL over the last 20–30 years and both drugs induce a high rate of partial remissions, it is also recognized that no treatment available to date has resulted in improvement of the natural history of this disease. The overall median survival time has remained at about 6 years.

Fludarabine

As mentioned earlier, fludarabine[104–106] has proven to be an extremely effective agent for those patients who have failed therapy with an alkylating agent. The potential role of fludarabine as front-line therapy for CLL is under active investigation in a large multi-institutional study in which patients are randomized to receive fludarabine or chlorambucil, or a combination of these two drugs. In this NCI-sponsored study led by Cancer and Leukemia Group B, NCI-Canada's Clinical Trials Group, South West Oncology Group, and Eastern Cooperative Oncology are participating. A single institution-based experience in a relatively small number of patients demonstrated a complete remission rate of 33% among previously untreated patients with active CLL.[104] It will be very important to determine whether the patients achieving complete remission with fludarabine have a prolonged survival—either equal to or better than the survival rate for stage 0 (low-risk group) patients.

2-Chlorodeoxyadenosine

2-Chlorodeoxyadenosine (2-CdA) is also a purine analogue like fludarabine, and is resistant to the action of adenosine deaminase. This drug has proven to be extremely effective in inducing lasting remissions in hairy cell leukemia. 2-CdA has been used in a relatively small number of CLL patients, mostly previously treated and refractory to alkylating agents.[154,155] The preliminary results are very encouraging and controlled clinical trials in previously untreated CLL are being planned; the results of such studies will have an impact on the future therapies of this disease. 2-CdA is given by a 2-hour intravenous infusion at a dose of 0.12–0.14 mg/kg/day for 5 days every month. Most patients obtain maximally attainable response after 4–6 months of therapy. Myelosuppression and immunosuppression are the major toxicities of 2-CdA.

Pentostatin

Pentostatin (deoxycoformycin) has a chemical structure somewhat similar to that of 2-Cda and is a potent inhibitor of adenosine deaminase. This drug also seems to offer benefit[156,157] to previously treated CLL patients, but it is used less often in CLL than fludarabine or 2-CdA.

Bone Marrow Transplantation as Therapy

With the recognition that CLL is being diagnosed today in increasing numbers in patients in the 35–50 age group, even a relatively long survival outlook of 6–10 years is not satisfactory for people this young. Simultaneously, we are developing an

increasing level of expertise in performing bone marrow transplantation and in managing the various complications of this therapy in several other types of human malignancies. In the United States[158] and in Europe,[159] results of small trials have been reported that reveal that bone marrow transplantation is a feasible approach in properly selected groups of CLL patients. An allogeneic bone marrow transplantation is preferred if a sibling is available as an HLA-compatible donor. In the absence of a compatible donor, autologously harvested marrow (after intensive chemotherapy-induced maximal reduction of leukemic cell mass in the patient's bone marrow) is reinfused following myeloablation with massive doses of chemotherapy. The preliminary results are encouraging and in the next few years we expect to learn more about this interesting and promising therapy.[158,159]

Monoclonal Antibodies

Immunotherapy with monoclonal antibodies either alone or conjugated with toxins or radioisotopes are becoming very attractive treatment measures[160] because of their potential of killing only the targeted tumor cells and sparing the normal hematopoietic cells. However, so far these approaches in CLL have been in early experimental stages; most published reports demonstrate that such therapies are feasible.

Anti-B4 (CD19) blocked ricin,[161,162] an immunoconjugate, has shown some activity in phase I studies but there was an associated risk of development of antibodies to the murine monoclonal antibody being used. CAMPATH-1H monoclonal antibody was developed by "humanization" of rodent variable immunoglobulin regions with human immunoglobulin gene sequences that present fewer xenogenic-peptide sequences and are thus less immunogenic.[163] Our own limited experience with CAMPATH-1H has been extremely promising[164] and we are continuing these studies on a larger number of patients.

T-CELL CLL

The T-cell variant of CLL, a rare form accounting for only 2-5% of all CLL cases, is briefly reviewed here. The diagnosis is suspected when confirmation of a lymphocytosis in blood and bone marrow is accompanied by phenotype analysis of blood lymphocytes revealing a preponderance of T cells.[165] Within this rare group of diseases there is considerable heterogeneity with respect to clinical features, clinical course, and phenotypic markers.[165] The most benign end of the spectrum consists of large granular lymphocytic leukemia associated with neutropenia, a T8+, T4−, T3+ phenotype (suppressor T lymphocytes), multiple autoantibodies (rheumatoid factor, antinuclear antibodies), splenomegaly, and absence of lymphadenopathy. Cytogenetic, immunologic, and functional studies indicate that this disease results from a clonal proliferation of immature NK cells.[166] The clinical course is variable but in most cases is rather indolent. Treatment is generally based on corticosteroids with the addition of alkylating agents if there is evidence of disease progression. The T4 CLL variant[167,168] usually affects patients <40 years of age and is associated with hyperlymphocytosis and marked generalized lymphadenopathy, frequently involving the skin and central nervous system. The lymphocyte morphology reveals small, mature-appearing cells with a notched nucleus that lacks a nucleolus and without cytoplasmic granules. The phenotype is T3+, T4+, and T8−. The clinical course is aggressive, the response to the usual cytotoxic therapy is inadequate, and overall survival is <2 years. Sézary syndrome is the leukemic manifestation of cutaneous T-cell lymphoma. The lymphocyte phenotype is $CD4^+$, $CD2^+$, $CD3^+$, and $CD5^+$. Treatment is directed to the underlying lymphoma. Adult T-cell leukemia, which is seen in certain areas of Japan, the Caribbean, and in the southeastern United States, is an HTLV-1 associated disease and bears little relation to CLL.

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Hairy Cell Leukemia

84

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INTRODUCTION

Hairy cell leukemia (HCL), or leukemic reticuloendotheliosis, is a rare chronic lymphoproliferative disorder first described in the literature as a distinct clinicopathologic entity in 1958 by Bouroncle et al.[1] The disease is characterized by circulating B lymphocytes that display prominent cytoplasmic projections and have a characteristic pattern of infiltration in the bone marrow and spleen. Because of this characteristic pattern, the original name of leukemic reticuloendotheliosis was replaced in 1966 by the more descriptive name, hairy cell leukemia.[2] Patients tend to be elderly, often presenting with pancytopenia, splenomegaly, or recurrent infections. In recent years, interferon (IFN)-α, 2'-deoxycoformycin (dCF), and 2-chlorodeoxyadenosine (2-CdA) have been shown to be capable of regularly inducing remissions in HCL. These clinical advances have revo-

EXHIBIT E

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Clinical Manifestations and Staging of and Therapy for Non-Hodgkin Lymphomas

81

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INTRODUCTION

In 1993 an estimated 43,000 new cases of non-Hodgkin lymphoma (NHL) were diagnosed in the United States.[1] It is estimated that these will account for 20,500 deaths. Although these cases account for <5% of all newly diagnosed cancers, they are among the five leading causes of cancer mortality in young men and women and may therefore have a disproportionate social and economic impact. The study of lymphomas has advanced our understanding of the function of the immune system, particularly in terms of lymphocyte development. Exciting discoveries have allowed new insights into the origins of these malignancies. Finally, with the introduction of combination chemotherapy >20 years ago,[2,3] it has become clear that these malignancies are curable. NHLs have thus become a proving ground for new chemotherapeutic agents. New chemotherapy dosing and administration schedules have validated previously described concepts of cell kinetics and have improved on early treatment results and allowed successful treatment even of patients with advanced or relapsed disease.

Because the goal in treating lymphomas is most often cure rather than palliation, a meticulous approach to diagnosis and staging is mandatory. The diagnosis of lymphoma is among the hardest for a pathologist to make. Fortunately, advances in immunophenotyping, cytogenetics, and molecular biology can provide help in situations in which diagnosis was previously impossible.[4] Since an accurate diagnosis may determine prognosis as well as therapy, any uncertainty should lead to review by a consulting pathologist or to a repeat biopsy if required. New drugs are currently under evaluation, along with other promising modalities, including biologic response modifiers, radioimmunoconjugates, and bone marrow transplantation (BMT). In addition, the use of hematopoietic growth factors as a means of increasing dose intensity is under active exploration. These, hopefully, will build on our past successes and improve our treatment results.

EPIDEMIOLOGY AND ETIOLOGY

The prevalence of NHLs shows wide geographic variations throughout the world. There is as much as an 8–10-fold range of occurrence, with the maximum rate reported in the Western developed countries.[5] The incidence rises steadily with age, especially beyond age 40.[6,7] However unlike Hodgkin disease, no peak in incidence of NHL exists for young adults. Males are affected more often than females, the ratio being 1.5:1.0, and in the United States the incidence is approximately twice as great among whites as among blacks. The differences in gender and racial incidence may reflect occupational risks that are experienced preferentially by the more frequently affected groups. The incidence of NHL has been steadily rising, not only in the United States but also worldwide.[6,7] Over the past 20 years, the incidence rates for NHL have increased 3–4% each year. This rate of increase is greater than for any other cancers except melanoma and lung cancer in woman. Although the reason for the increase in incidence is not completely understood, it is partly related to an increase in acquired immunodeficiency syndrome (AIDS)-associated lymphomas. This is supported by Surveillance Epidemiology and End Results (SEER) data that show the largest increase in NHL incidence is in San Francisco. As utilization of standardized reporting and classification methods for NHL increases, perhaps more information regarding true differences in the incidence of NHL will be obtained.

No common etiologic agent can be associated with all cases of NHL. However, a number of genetic, immunologic, and environmental factors have been associated with some cases. Several families have now been described with an unusually large number of lymphoproliferative diseases among their members.[8] A family history of NHL or other lymphoproliferative disorder is associated with a markedly increased risk of NHL. One category of familial NHL involves sibling pairs, with mostly young males affected. Many of these patients have had extranodal lymphoma at diagnosis. Another category of familial NHL

affects adult siblings, and a third category has included families with adult NHL occurring in more than one generation. When such a familial incidence is noted, the entire family should be screened for an underlying immunodeficiency syndrome.

Although many families with an increased incidence of lymphoproliferative diseases do not have an underlying immunodeficiency syndrome, there are several immunologic disorders that do predispose to an increased incidence of lymphomas. Rare immunologic or inherited disorders that are associated with an increased incidence of NHL include ataxia-telangiectasia, Wiskott-Aldrich syndrome, common variable immunodeficiency syndrome, Bruton-type agammaglobulinemia, and Chédiak-Higashi syndrome.[9,10] Of particular interest is the X-linked lymphoproliferative syndrome, a rare condition, described in 1976 by Purtilo,[11] in which families exhibit impaired cellular immunity to the Epstein-Barr virus (EBV) in an X-linked recessive pattern. The affected male members of these families have uncontrolled EBV infections expressed as fatal infectious mononucleosis, NHL, or acquired agammaglobulinemia.[12,13]

Many cases of NHL are related to iatrogenic causes. The frequency of NHL is high in patients who have been treated for Hodgkin disease.[14] Iatrogenic immunodeficiency also leads to an increased risk of developing NHL. This risk applies to patients who are receiving immunosuppressive agents to prevent transplant rejection or for treatment of other conditions. The incidence of NHL in recipients of renal allografts has been approximately 40–100 times greater than expected. These lymphomas occur within 2–3 years of transplant on average, but may occur within a matter of months. The central nervous system is a frequent site of involvement, and other extranodal sites of presentation are common.[15–17] The allograft itself is a frequent site of microscopic or macroscopic disease. These tumors display a wide spectrum of histologic appearance and may be monoclonal or polyclonal proliferations of B lymphocytes.[18] The use of the monoclonal antibody OKT3 has been associated with an increased risk of lymphomas after cardiac transplantation.[19] An increased risk of lymphoproliferative syndromes after allogeneic BMT has been described as well.[20,21] There seems to be a correlation with HLA-mismatched and T-cell-depleted transplants, presumably as a result of increased immunosuppression. Lymphoma risk in persons receiving immunotherapy for other clinical conditions appears to be less pronounced.[22]

It is now clear that many, if not all, lymphomas that occur in the setting of acquired or congenital immunodeficiency are caused by EBV.[17,23,24] This virus, which infects B cells, is normally suppressed by T-cell-mediated immune mechanisms. When T-cell deficiencies, either congenital or acquired, occur, EBV-infected B cells can proliferate unchecked. Initially a polyclonal proliferation takes place; however, one clone eventually can escape immune surveillance to become autonomous. Various cytogenetic abnormalities have been associated with this second step in differentiation to a monoclonal proliferation.[25]

A wide range of disorders with impaired immunity have also been associated with an increased risk of lymphoma. Rheumatoid arthritis,[26] Sjögren-Larsson syndrome,[27] Hashimoto thyroiditis,[28] systemic lupus erythematosus,[26] and celiac sprue[29] have all been associated with this increase in incidence of lymphomas. The patients with celiac sprue often present with primary gastrointestinal lymphoma, the distal ileum being the most frequent site of involvement.[30]

Environmental factors may play a part in the induction of lymphomas. A small but significant increase in NHL incidence has been demonstrated in patients receiving radiation for ankylosing spondylitis,[31] as well as in Japanese atomic bomb survivors who had been exposed to >100 cGy.[32] Several surveys of cancer risk in relationship to occupational associations have suggested that some occupations may be associated with NHL.[33–35] These include vinyl chloride workers, anesthesiologists, rubber production workers, leather workers, and road transport workers. These exposures, however, are related to only a small percentage of lymphomas. An increase in the incidence of lymphoma among farmers has been noted in several epidemiologic studies. Use of the herbicide 2,4-D has been associated with a two- to eightfold increase in NHL. Risk of NHL can be directly correlated with duration of herbicide exposure. Exposure to hair dye has also been linked to the development of NHL.[34] It has been estimated that hair dye may account for as much as 20% of NHL in women.

Several lymphomas have now been associated with a viral agent; however, such an association has not been clearly delineated for most types of lymphoma.[36] In humans there is a strong association between EBV and Burkitt lymphoma in Africa, but the association is not as strong for Burkitt lymphoma cases diagnosed in the United States.[37] Another lymphoproliferative syndrome that has been associated with a viral agent is adult T-cell leukemia/lymphoma, which is endemic in southwestern Japan, the Caribbean, and also the southeastern United States.[38,39] The virus isolated from this type of lymphoproliferative disorder is a type C RNA retrovirus—the human T-cell leukemia/lymphoma virus-1 (HTLV-1).[40] Other viruses associated with lymphomas include HTLV-3 (human immunodeficiency virus-1 [HIV-1] the causative agent of AIDS,[41] and HTLV-4 (HIV-2), which is a similar retrovirus isolated from West African patients with AIDS.[42] A recently discovered agent, HTLV-5, may be associated with certain subtypes of cutaneous T-cell lymphoma, although questions remain regarding its actual role.[43]

Both host and environmental factors seem to be important in the etiology of NHLs. Host factors, including genetic predisposition and underlying immunodeficiency syndromes, may set the stage for the development of lymphoproliferative syndromes. Environmental agents such as chemicals, immunosuppressive drugs, radiation, or viruses may also predispose to lymphoma development. Further analysis of this complex group of diseases may uncover how these factors may interact to initiate lymphoproliferative disorders.

The large number of classification systems for NHLs has led to considerable confusion for both pathologists and practicing physicians when attempts were made to correlate diagnoses from one classification system to another.[44–58] The various classification systems are discussed in detail in Chapter 80. In order to relieve the confusion in terminology that resulted from these different systems of classification, an international multiinstitutional study was undertaken in an attempt to assess the clinical applicability and reproducibility of the six major classification systems for NHL.[59] Pathologic material from 1,175 newly diagnosed patients seen at four institutions between July 1, 1971 and December 31, 1975 was reviewed and classified by six expert pathologists, each a proponent of one of the major classification systems. Six other hematopathologists were selected to review the same slides, and they also independently reviewed and classified all the cases according to all six systems of classification. The results of the analysis led to the development of a working formulation of the NHLs for clinical purposes, which is described in Chapter 80. It was concluded that all six classification symptoms were valuable and comparable in reproducibility and clinical correlations. The working formulation also confirmed the significance of follicular architecture independent of cell type. Ten major cell types were identified, along with a miscellaneous category, and subtypes were described that allowed comparisons between one system and another.

CLINICAL MANIFESTATIONS

Low-Grade Histologies

The subset of NHL with low-grade histology consists of the small lymphocytic, follicular small cleaved cell, and follicular mixed categories in the working formulation.[59] Because of their

Table 81-1. Characteristics of the Major Histologic Subtypes of the NHLs[a]

Histologic Subtype[59]	Cases (%)	Distribution by Stage (%)				Symptom Status (%)	Bone Marrow Involvement (%)	Median Survival (yr)	Cure with Aggressive Chemotherapy Possible
		I	II	III	IV				
Small lymphocytic	4	3	8	8	81	20	71	5.8	No
Follicular small cleaved	22	8	10	16	66	20	51	7.2	No/rare
Follicular mixed	8	15	12	28	46	20	30	5.1	Uncertain
Follicular large cell	4	15	12	15	58	27	34	3.0	Probable
Diffuse small cleaved	7	9	19	12	60	18	32	3.4	Probable
Diffuse mixed	7	19	26	13	42	21	14	2.7	Yes
Diffuse large cell	20	16	30	10	44	28	10	1.5	Yes
Immunoblastic	8	23	29	16	33	32	12	1.3	Yes
Lymphoblastic	4	7	20	2	72	27	50	2.0	Yes
Small noncleaved	5	13	21	9	57	14	14	0.8	Yes

[a] A miscellaneous group comprises the remaining 12% of NHLs and are not included in this table. This category includes composite NHL, mycosis fungoides, histiocyte lymphoma, extramedullary plasmacytoma, and unclassifiable NHLs.

natural history and response to therapy they have often been referred to as "good prognosis" or "favorable prognosis" lymphomas. These histologies make up 23–43% of newly diagnosed NHLs[60–64] (Table 81-1). Although not uncommon in the third and fourth decades, these lymphomas occur most commonly in middle-aged patients and the elderly, with a median age at diagnosis of 50–60 years.[60–64] Bone marrow involvement is commonly seen at presentation, and ≥50–80% of patients will present with stage III or IV disease.[62,65–67] With few exceptions these lymphomas are composed of B lymphocytes.[68] By using flow cytometric κ-λ analysis or Southern analysis, circulating monoclonal B lymphocytes have been detected in ≤78% of these patients.[69,70] Numerous primary and secondary cytogenetic abnormalities have been described in patients with low-grade histologies. The specific abnormality, t(14;18) (q32; q21) has been seen in most patients with follicular lymphomas.[71–75] This cytogenetic abnormality is associated with deregulation of the *bcl-2* proto-oncogene. This results in overexpression of the bcl-2 protein, which is known to inhibit programmed cell death.[76]

Although median survivals >7–8 years are reported from several series,[77–82] the low-grade lymphomas should be considered fatal malignancies. The median survival of a group of 83 patients with low-grade lymphoma who were managed initially with observation alone was 11 years.[80] With time, these lymphomas often transform into a more aggressive histologic pattern; when patients have a repeat biopsy during the course of their disease, 28–44% may show evidence of such a transformation.[61,80,83–86] Rates of such histologic transformation have been >70% when patients with residual disease are examined at autopsy.[87,88] These transformed lymphomas may be quite aggressive and poorly responsive to chemotherapy.[61,82]

Intermediate-Grade Lymphomas

Intermediate-grade lymphomas considered in the working formulation include follicular large cell, diffuse small cleaved, diffuse mixed, and diffuse large cell. Since clinically there is very little distinction between tumors labeled diffuse large cell and those classified as immunoblastic, that category is also included in this discussion. As can be seen in Table 81-1, intermediate grade tumors make up approximately 50% of NHLs. The most common are the diffuse large cell lymphomas.

Follicular large cell lymphomas are closely related to the other follicular lymphomas. They are the least common of the follicular lymphomas, making up 4% of the cases on which the working formulation was based. Like all other subtypes of follicular lymphoma, these tumors tend to be widely disseminated at diagnosis, and a minority of patients have systemic symptoms. Follicular large cell lymphomas can be identified by a variety of criteria, of which perhaps the most useful is that of Mann and Berard,[89] who proposed that a distinction between follicular mixed and follicular large cell lymphoma should be made when there are >15 large cells per high-power field. The criteria for separating follicular mixed and follicular large cell NHL are not the same among the various classification systems, and the distinctions are difficult to reproduce among pathologists. This makes it difficult to interpret clinical trials involving patients with follicular large cell lymphoma. Follicular large cell lymphomas are of B-cell origin, but it must be remembered that reactive T cells can infiltrate the tumor and cause confusion in immunophenotyping.[90] The most common chromosomal abnormality seen in follicular large cell lymphoma is the t(14;18) (q32;q21) translocation that is characteristic of other follicular lymphomas. With the usual cytogenetic methods, this translocation is found in approximately 50% of follicular large cell lymphoma cases. It can be accompanied by a variety of other cytogenetic abnormalities.[91]

Follicular large cell lymphoma seems to have a more aggressive natural history than the other follicular lymphomas, particularly in patients with advanced-stage disease.[92–94] This tumor is more likely to progress quickly when patients are observed without therapy. The greater number of large cells in the tumor has led to some enthusiasm for the treatment of these patients with aggressive chemotherapy regimens in hopes of cure, although the percentage of large cells may not have prognostic influence.[86] Whether this tumor can be cured in a significant number of patients with aggressive chemotherapy regimens remains a point of controversy.

Diffuse small cleaved cell NHL is the diffuse counterpart of follicular small cleaved cell lymphoma.[95] This tumor has been diagnosed less commonly in the United States over the last several years. As outlined in Table 81-1, patients with diffuse small cleaved cell lymphoma generally have disseminated disease without systemic symptoms. This tumor usually represents a B-cell neoplasm, although occasional patients with peripheral T-cell lymphoma will be classified in this subcategory. The natural history of tumors in this category varies considerably. When patients with true lymphoblastic lymphoma are carefully excluded, the typical course of patients with this lymphoma subtype is usually one of response to therapy with frequent relapses. It is unclear whether these lymphomas are curable with standard chemotherapy, although the working formulation study[59] suggested that some proportion of these patients can be cured. However, many oncologists in the United States would believe that patients with small cleaved cell lymphoma should be managed similarly regardless of the degree of follicularity in the tumor. We favor a more aggressive approach and treat these patients initially much as we treat those with large cell lymphoma.

Diffuse mixed lymphoma is one of the most varied subtypes included in the working formulation. These tumors have a distribution similar to that of large cell lymphomas with respect to the percentage of patients with localized and with disseminated disease at diagnosis. Most patients will not have systemic symptoms at diagnosis. With the exception of lymphoblastic lymphoma, diffuse mixed lymphomas represent the subgroup with the highest proportion of T-cell lymphomas. These lymphomas are generally post-thymic or peripheral T-cell lymphomas. No single chromosomal abnormality is characteristic. It is important to note that peripheral T-cell lymphoma of the diffuse mixed cell type can easily be misdiagnosed as mixed-cellularity Hodgkin disease.

We have found the clinical course of patients with diffuse mixed lymphoma to be so similar to that of patients with large cell lymphomas that there is no useful distinction with regard to staging and therapy. Patients with diffuse mixed lymphoma have complete responses to therapy, and the proportion of long-term survivors is approximately the same as with diffuse large cell or immunoblastic lymphoma.

Diffuse large cell and immunoblastic lymphomas are aggressive neoplasms. These tumors present in a localized (i.e., stage I) manner approximately 20% of the time, and disease is confined to one side of the diaphragm (i.e., stage I or II) approximately 50% of the time. Disseminated extranodal disease (i.e., stage IV) is seen least frequently in these types of lymphomas. Approximately one in three patients have systemic symptoms. These tumors can have either a B- or a T-cell immunophenotype; in the United States approximately 80–90% of the patients will have B-cell lymphomas and the remainder will have the peripheral T-cell immunophenotype.[96,97] The cytogenetic abnormality t(14;18) (q32;q21) is the one most frequently seen in these subtypes, although a wide number of other cytogenetic abnormalities have been described.[74,98] The significance of the particular pattern of cytogenetic abnormalities remains uncertain, but it has been proposed that the pattern might have prognostic significance. For example, patients with abnormalities involving chromosome 17 or 7 have poor survival,[98–100] and patients with abnormalities involving chromosome 2 have improved survival.[98]

Patients with diffuse large cell or immunoblastic lymphoma have aggressive disease, which progress rapidly without therapy. However, with therapy these subtypes of lymphoma are curable in a significant proportion of patients. Most of those who present with localized disease are curable, and even patients with widely disseminated, symptomatic disease can be cured in a significant proportion of cases. This particular subtype of lymphoma can occasionally be misdiagnosed as an undifferentiated carcinoma. When patients present with undifferentiated malignant neoplasms, the diagnosis of large cell lymphoma should be considered because of the considerable therapeutic implications.[101]

High-Grade Lymphomas

The working formulation for NHL clinical usage divides the high-grade NHLs into three major categories: malignant lymphoma-large cell immunoblastic, malignant lymphoma-lymphoblastic, and malignant lymphoma-small noncleaved cell.[59] These high-grade lymphomas behave in a clinically aggressive manner if left untreated; however, with modern therapy long-term disease-free survival is possible. Overall, these lymphomas represent approximately 12–18% of all NHLs. Most cases of high-grade NHLs present in young patients and almost half of childhood NHL falls into this category. Other histologies in the working formulation that manifest aggressive biologic characteristics, such as advanced-stage peripheral T-cell lymphoma, may also be considered in this high-grade category and are discussed here.

The category of malignant lymphoma-large cell immunoblastic lymphoma would be considered diffuse "histiocytic" lymphoma in the Rappaport scheme, and thus diffuse histiocytic lymphoma was subclassified in the working formulation into large cell and large cell immunoblastic categories. Because of a small but significant difference in survival and certain morphologic distinctions, these cases were separated from the diffuse large cell type in the working formulation.[59] However, the studies, although showing survival trends in the same direction, failed to demonstrate a statistical difference in survival.[102] Furthermore, because the large cell and large cell immunoblastic categories share many clinical features, the intermediate- and high-grade categories of the working formulation have been controversial. Because of the clinical similarities most oncologists would evaluate and treat immunoblastic NHL in a manner similar to that used for diffuse large cell NHL, which has been considered under the intermediate-grade lymphomas.

Lymphoblastic NHL was originally included in the diffuse, poorly differentiated lymphocytic lymphomas of the Rappaport classification; however, on clinical and pathologic grounds it is clearly a distinct entity.[103] In 1975 Barcos and Lukes[104] used the term *convoluted lymphocytic lymphoma* to describe this clinical subgroup, seen predominantly in young males who present with a large mediastinal mass and progress rapidly to disseminated lymphoma. Nathwani et al.[105] subsequently recognized both convoluted and nonconvoluted cell types in these lymphomas and classified them as lymphoblastic lymphoma. This lymphoma shares many of the features of T-cell acute lymphocytic leukemia (T-ALL) and may be a close variant. In contrast to T-ALL, lymphoblastic NHL occurs in a slightly older age group with a peak incidence in the second decade of life, and males outnumber females in approximately a 2:1 ratio.[106]

Most patients (approximately 50–75%) present with a mediastinal mass, and emergent symptoms related to superior vena cava syndrome or tracheal obstruction often are initially present.[107] Although the disease occasionally appears to be localized at diagnosis, it evolves rapidly and is characterized by disseminated systemic involvement. Bone marrow involvement is present at diagnosis in approximately 30% of cases; however, as the disease progresses, ≤80% of patients eventually develop bone marrow and peripheral blood involvement.[108] Because of this clinical overlap, the boundaries between T-ALL and lymphoblastic lymphoma can be indistinct. The frequently used St. Jude Children's Hospital staging system for childhood NHL includes patients in the lymphoblastic lymphoma category if they have <25% blasts in their bone marrow.[109] However, other systems use a 10% lymphoblast cut-off.[110] In addition to the marrow and blood, central nervous system involvement is also detected in approximately one-third of patients at some time during the clinical course.

The small noncleaved lymphomas (SNCLs) of the working formulation were designated as undifferentiated lymphomas in the Rappaport classification. Within this subgroup a histologic distinction between Burkitt and non-Burkitt lymphoma can be made based on the degree of cellular pleomorphism and the proportion of cells with a single large nucleolus. However, the importance of this distinction clinically remains controversial.

"Endemic" regions of the world, which include Africa and New Guinea,[111] have a relatively high incidence of SNCL—5–10 cases per 100,000 children, with few cases in the adult population. These cases are associated with significantly elevated antibody titers to a variety of EBV antigens, and 80–90% of the tumors contain multiple copies of the EBV DNA genome.[112,113] The "sporadic" regions for SNCL include most of the United States and Europe. The incidence is lower in the sporadic regions, with two to three cases per million children, which account for approximately 1–2% of NHLs in all age groups.[114] The association with EBV is not as clear in the sporadic as in the

endemic cases of SNCL. Most Burkitt lymphomas (75%) have a chromosomal translocation identified as a t(8;14), with another 20% having a t(8;22) and 8% having a t(8;2).[99,115] The common features of these translocations are involvement of the *c-myc* oncogene located on chromosome 8 and juxtaposition of the protein-coding region of this gene with sequences from one of the immunoglobulin gene loci. Chromosome 14 contains the immunoglobulin heavy chain locus, while chromosomes 2 and 22 contain the immunoglobulin κ and λ light chain loci, respectively.

The clinical features of endemic and sporadic cases of SNCL are somewhat different. The African endemic SNCL most often presents as large extranodal tumors affecting the bones of the jaws and abdominal viscera. Occasionally patients can present with isolated tumors of the thyroid, skin, breasts, testes, or long bones. Retroperitoneal or extradural tumors can cause paraplegia, either by vascular compromise or by direct spinal cord invasion. Involvement of the central nervous system is an unusual presenting feature, but it becomes increasingly common after relapse and may be manifested by cranial nerve palsies. The mean age of African patients with SNCL is 7 years, with a 2:1 male/female ratio. Patients with sporadic SNCL present most of the time with intra-abdominal tumors, arising apparently from Peyer's patches in the ileocecal region or from the mesenteric lymph nodes. Patients often have bowel obstruction or perforation as an initial clinical presentation. The remainder of the sporadic cases present as tumors involving the ovaries, kidneys, retroperitoneum, or peripheral lymph nodes or as diffuse bone marrow involvement. The mean age in the sporadic cases is slightly older, 11 years, with the same male/female ratio.

The final category that we would classify as high-grade lymphoma on the basis of clinical parameters consists of peripheral T-cell lymphomas, including those of advanced stage or with large tumor burden. Although not all previous studies have found peripheral T-cell lymphoma to be an important prognostic characteristic,[97,116,117] several recent studies of uniformly treated patients have found this to be the case.[95,96,118]

STAGING

One of the important concepts in managing patients with malignant disease is the concept of staging. This implies an evaluation of patients that classifies them into one of multiple categories to allow therapeutic decisions and/or accurate determination of the prognosis. Because lymphoma patients have illnesses that sometimes can be cured and in most other cases can be palliated with a variety of different chemotherapeutic and radiotherapeutic approaches, staging these patients is the most important initial responsibility of the physician after the diagnosis has been firmly determined. The importance, as the initial step, of confirming the histologic diagnosis by having an expert hematopathologist review the slides cannot be too strongly emphasized.

Staging is often considered to be based on determination of the sites of involvement by disease. However, there are other staging systems that can be used. For chronic lymphocytic leukemia the Rai system divides patients into groups based on the sites of involvement and the extent of marrow failure.[119] A popular staging system for multiple myeloma depends on sites of involvement, hypercalcemia, extent of protein abnormalities, and signs of marrow failure.[120] The most popular staging system for patients with lymphoma, often referred to as the Ann Arbor staging system,[121] was originally developed for patients with Hodgkin disease, and not those with NHL. The differences between these disorders make application of the Ann Arbor system to NHL patients not always simple. However, it remains the most popular system in use for patients with most types of NHL.

Table 81-2. Evaluation and Staging of Patients with NHL

Confirmation of histologic diagnosis
Careful history and physical examination
Routine studies (e.g., hemogram, chemistry profile, chest radiograph)
Bone marrow biopsy
Imaging studies to look for occult sites of involvement (e.g., CT scan, gallium scan, lymphangiogram, abdominal ultrasound)
Other tests as indicated by results of above (e.g., biopsy of suspicious area)

The Ann Arbor system assigns patients to stage I (disease confined to one lymph node site), stage II (disease confined to lymphatic tissue in more than one site but on only one side of the diaphragm), stage III (disease confined to lymphatic tissue or spleen but on both sides of the diaphragm), or stage IV (bone marrow involvement, liver involvement, or any other site of extranodal disease with widespread lymphoma). When patients present with localized extranodal disease (e.g., a disease originating in and confined to the stomach), they are designated as having stage IE disease, and patients with localized extranodal disease and regional lymph node involvement would be designated as having stage IIE. Some physicians would designate widespread lymphatic disease and one localized site of extranodal involvement other than the liver or bone marrow as stage IIIE, whereas others would classify such patients as having stage IV. Patients are further subdivided in the Ann Arbor classification depending on whether they have the systemic symptoms of fever (i.e., >38°C with no other cause), weight loss (i.e., >10% body weight in 6 months), or drenching night sweats. When the Ann Arbor classification is applied to patients with Hodgkin disease, there is a distinction made between clinical staging (examination, laboratory, and imaging studies) and pathologic staging (including the results of staging laparotomy). Because staging laparotomies are rarely done in NHL patients, this distinction is less useful for NHL.

Table 81-2 presents one approach to the staging of NHL patients. It cannot be stated too firmly that the first step is to confirm the histologic diagnosis by having the slides reviewed by an experienced hematopathologist. This should be followed by a careful history and physical examination to identify potential sites of involvement and the presence or absence of systemic symptoms. Findings from the history and physical examination may direct further biopsies to document a high stage. A variety of standard laboratory studies, including a hemogram, chemistry profile, chest radiograph, and bone marrow biopsy, should be performed on all patients. The first three tests also aid in identifying possible areas for documentation of advanced disease, and a positive bone marrow biopsy is especially important in that it would always make the patient stage IV. A wide variety of imaging studies beyond the use of the chest radiograph have been used with NHL. These include the computed tomography (CT) scan, gallium scan, lymphangiogram, ultrasound scan, and magnetic resonance imaging (MRI). Of the multiple other imaging studies available, radionuclide bone scans can sometimes be helpful. The most important reason for the more complex imaging studies in patients being staged for lymphoma is the possible detection of occult intra-abdominal disease. Staging laparotomies will be done only rarely in these patients, and physical examination, laboratory studies, and routine radiographic studies have limited value in identifying intra-abdominal adenopathy or organ involvement. Perhaps the most important advance in this regard was the development of the CT scan, which is fairly simple to use and has a high accuracy in identifying sites of lymphoma involvement.[122] CT scans are particularly useful in identifying high periaortic, mesenteric, and splenic hilar nodes, which are not seen by lymphangiography.[123]

Although performed with decreasing frequency, lymphangiography has been the standard test for identification of involve-

ment of periaortic lymph nodes by Hodgkin disease or NHL. This procedure has a high accuracy,[124] but it has been replaced by CT scans to a significant degree. Ultrasonography provides a comparatively easy and noninvasive imaging approach, which can be used to study a number of sites of the body in addition to the abdomen.[125] It is worth remembering that ultrasound can be used to confirm or refute the suspicion of possible superficial adenopathy,[126] and endoscopic ultrasonography might extend the usefulness of this technique.[127] MRI offers a new approach to the imaging of patients with lymphoma; in addition to identifying usual sites of disease, MRI scans might be helpful in detecting bone marrow involvement without bone marrow biopsies.[128] In addition, MRI may prove to be a useful way to evaluate residual masses on CT scans after treatment.[129]

Of the radionuclide imaging techniques, gallium scans probably have the most utility in NHL patients. However, it must be remembered that this test is much more useful in patients with aggressive NHL than in those with indolent lymphomas, whose tumors are less likely to take up gallium.[130] When combined with single-photon emission computed tomography, gallium scans become especially useful.[131] Gallium scanning can be especially useful in re-evaluating patients after therapy, particularly those with residual, potentially fibrotic masses. This is true because gallium scanning reflects the metabolic activity of the tumor rather than anatomic findings. However, this technique is not perfect in that both false-positive and false-negative scans can occur.[132,133] The other radionuclide study that is often used in NHL patients is the bone scan. Although not recommended for routine use, this is a sensitive technique that can be used to identify bone marrow involvement in patients in whom it is suspected.[134]

A more subtle reason for careful staging of the lymphoma patient before therapy is to allow accurate determination of the response at the completion of therapy. This is often referred to as restaging. In general, all studies that gave abnormal results before therapy should be repeated at the completion of therapy. This will allow the accurate determination of a complete response. As noted above, some patients (particularly those with bulky mediastinal or retroperitoneal disease) are likely to have residual imaging abnormalities reflecting persistent fibrotic masses. In general, patients with stable, residual masses in sites of bulky disease, particularly if the mass has gone from gallium-positive to gallium-negative, should be followed without further therapy and treated as complete responders.[135] A new staging system for Hodgkin disease has been proposed in which patients with residual masses of uncertain significance are given the classification CR[u] to denote a complete remission of uncertain significance.[136] This designation is gaining usage for patients with NHL.

PROGNOSIS

A wide variety of factors have been determined to predict outcome in some groups of NHL patients, the most widely used prognostic factor being histologic subtype.[59,62] Although it should be remembered that the various histologic subtypes do not carry sharply different prognoses (e.g., some patients with follicular small cleaved cell lymphoma will have a poorer survival than patients with diffuse large cell lymphoma), this is still an important factor on which to base therapeutic decisions. Some other prognostic factors might be especially important only when histologic subtype is taken into account, and some would apply to all different subtypes of lymphomas. Prognostic factors can be divided into those related primarily to the patient (e.g., age, concomitant illnesses) and those related to the tumor itself (e.g., immunologic subtype, cytogenetic abnormalities). Of the patient-related prognostic variables, perhaps the most consistent is the poor outcome found with advanced age.[137–139] The apparent lack of importance of age in some series of patients with aggressive NHL is probably because few patients >60–65 years of age (at which point there seems to be a significant drop-off in favorable outcome) were actually treated.[140,141] The general health of patients as reflected in their performance status is an important factor in survival for many malignancies and seems to be important also in NHL patients.[142] Obviously, the existence of serious concomitant illnesses such as lung or heart disease might greatly limit the drugs that can be used and thus alter the physician's ability to treat the patient effectively. Patients who have failed previous chemotherapy regimens have a poor outlook with second-line therapy,[143] as do patients who have undergone progression from a low-grade to a more aggressive histology.[61,82–84,144]

Tumor-related variables such as bulkiness[142,145–150] or high growth fraction[151–154] are associated with a poor prognosis. The significance of stage in treatment outcome presumably is a reflection of both these variables.[148,155] Similarly, the adverse prognosis associated with an elevated serum lactate dehydrogenase (LDH) level reflects bulky tumor and/or particularly rapid growth.[145,146,148,150,156,157] More recently, the serum level of β_2-microglobulin has been identified as a prognostic factor not only in multiple myeloma but also in NHL.[158]

The influence of T-cell versus B-cell phenotype is controversial. Some reports have shown no difference in outcome related to phenotype.[97,159–161] However, a number of other investigations have noted a significantly poorer outcome in patients with a T-cell phenotype.[95,96,118,162] Certain cytogenetic abnormalities have also been associated with a poor outcome.[74,98,163] In addition, specific sites of tumor involvement, especially the bone marrow,[147,150,155] but also other sites such as the gastrointestinal tract,[147] have been identified as significant adverse prognostic factors. Several studies have shown that the rate of response of the tumor to initial therapy is highly predictive of outcome, with more rapidly responding patients having better outlooks.[164–166] This presumably is a direct reflection of the sensitivity of the tumor to the treatment being administered.

Recently, results from the International non-Hodgkin's Lymphoma Prognostic Factors Project[167] have been published. This study analyzed results of >3,000 patients with aggressive NHL in North America and Europe. An International Index was developed based on age, tumor stage, the number of extranodal sites of disease, performance status, and serum LDH. The influence of age (≤60 versus >60 years) was highly predictive, with elderly patients having significantly poorer outcomes. Therefore, an Age-Adjusted International Index was developed (Table 81-3) for patients ≤60 of age. This index was based on three variables: stage, serum LDH, and performance status. These models were more accurate than the Ann Arbor stage[121] in predicting survival, and these indexes are likely to be widely used in the future.

Table 81-4 outlines the other prognostic factors discussed above. It should be remembered that prognostic factors are useful for transmitting to patients some idea of their chances for successful therapy; they might also be useful in identifying

Table 81-3. Age-Adjusted International Prognostic Index for Aggressive NHL

Risk Factors (N)	Risk Group	5-yr Projected Survival (%)
0	Low	83
1	Low intermediate	69
2	High intermediate	46
3	High	32

Risk factor: (1) stage (I or II versus III or IV); (2) serum LDH (≤1 × normal versus >1 × normal); (3) performance status (0 or 1 versus 2–4).

(Modified from The International Non-Hodgkin's Lymphoma Prognostic Factors Project,[167] with permission.)

Table 81-4. Important Prognostic Factors in the Treatment of Patients with NHL[a]

Histologic subtype
Age
Other serious illnesses
Failed previous therapy
Histologic progression
Tumor bulk
Stage
Systemic symptoms
Performance status
Serum LDH
Serum β_2-microglobulin
Specific sites of involvement (e.g., bone marrow, gastrointestinal tract)
Immunophenotype
Cytogenetic abnormalities
Rate of tumor response

[a] See text for references.

patients who should receive one or another treatment. For example, patients with a particularly poor prognosis with primary therapy might be candidates for an alternate treatment such as BMT as part of the primary therapy.

THERAPY

Low-Grade Non-Hodgkin Lymphomas

No aspect of the optimal management of NHL patients is more controversial than that of patients with low-grade histologic subtypes. Although highly responsive to chemotherapy and radiation, most groups of patients with advanced disease have failed to show definite evidence of a plateau in their survival curves and have exhibited a continuous pattern of relapse.[81,86,168,169]

Interpretation of treatment results in these patients is difficult and should be undertaken with caution. Few studies are prospective or randomized, and many if not all are plagued by difficulties in classification. Small lymphocytic lymphomas, for example, are considered to be solid tumor counterparts of chronic lymphocytic leukemia.[170] A decision on whether to include those patients in lymphoma or chronic lymphocytic leukemia trials is often arbitrary. Other difficulties are seen when attempts are made to distinguish follicular mixed from diffuse mixed lymphomas or follicular mixed from follicular large cell histologies.

Follicular large cell lymphomas, although classified as belonging to an intermediate grade, are often grouped with low-grade histologies.[61,79,86,171,172] Since these lymphomas may respond differently to therapy, their inclusion in series containing low-grade histologies may influence results, and literature must be interpreted in this light.[61,172–174] How newer imaging technologies have influenced staging in more recent patient series is unknown, but their use certainly makes comparisons with older studies more difficult.

Localized Low-Grade Lymphomas

Although the treatment of patients with advanced low-grade lymphomas remains controversial, cure is sometimes possible with true stage I or minimal stage II. Table 81-5 shows treatment results for patients with localized low-grade lymphomas treated with radiation alone or in combination with chemotherapy.[171,172,175–180] A variety of radiation doses and treatment fields have been employed, but most series report 5-year disease-free survivals >50%. Differences in results may be accounted for in several ways.

Laparotomy has been used in staging some patients, generally those who would be more likely to have clinically occult disease outside the radiation ports, which may account for the superior disease-free survival (DFS) reported for laparotomy-staged patients in some series. Also, some series have reported superior DFS in patients treated with more extensive radiation fields. Despite a trend for improved DFS with more aggressive staging or more extensive radiation fields, it has been difficult to translate these findings into improved overall survival. Relapses have almost always occurred outside of radiation ports, however, which supports the concept that failure is often a result of inadequate treatment.

The question of whether adjuvant chemotherapy adds anything to the effectiveness of radiation alone is still unsettled. With few exceptions, the addition of chemotherapy has not been studied in a prospective randomized fashion. Furthermore, the length and type of chemotherapy employed has varied widely from study to study. Again, improvement in DFS has been reported with the addition of chemotherapy, but improvement in overall survival of patients with localized low-grade lymphomas is difficult to prove.

It seems that radiation alone can cure patients with low-grade NHL when disease is truly localized and all areas of disease are treated. The role of adjuvant chemotherapy and more extensive radiation is less clear, but improvements in DFS may indeed be translated into overall survival differences when these patients are followed long enough. We do not perform staging laparotomies. Patients are treated with three cycles of

Table 81-5. Radiotherapy for Localized Low-Grade NHLs

Reference	Patients (N)	Radiation Fields	Adjuvant Chemotherapy (N)	Results	Comments
171	57	IF	Yes (9)	FSC: 49% RFS, 5 yr FM: 44% RFS, 5 yr	5 patients with FSC received chemotherapy alone
172	124	IF, EF, or TLI	Yes (2)	54% RFS, 10 yr	Includes FLC
175	23	IF or EF	Yes (?)	55% RFS, 5 yr	Includes FLC
176	26	IF, EF, STNI, or TNI	No	83% RFS, 5 yr	Includes FLC
177	26	IF or EF	Yes, randomized (15)	54.6% RFS, 5 yr radiation alone 63.0% RFS, 5 yr radiation + chemotherapy	Includes FLC Includes FLC
178	190	IF	No	53% RFS, 12 yr	Includes FLC
179	25	IF or TLI	Yes, randomized (?)	55% CCR, median F/U 118 mo	
180	39	IF or EF	Yes (18)	28/39 CCR, median F/U 5 yr	

Abbreviations: IF, involved field radiation; EF, extended field radiation; STNI, subtotal nodal irradiation; TNI, total nodal irradiation; TLI, total lymphoid irradiation; RFS, relapse-free survival; CCR, continuous complete remission; FSC, follicular small cleaved; FM, follicular mixed; F/U, follow-up; FLC, follicular large cell.

Table 81-6. Initial Observation for Low-Grade Lymphomas

Reference	Patients (N)	Survival (%)	Median Time to Institution of Therapy	Comments
80	83	73 (10 yr)	3 yr	23% spontaneous regression
81	59	56 (5 yr)	20% still on watch and wait; median follow-up 88 months	Includes some intermediate histologies
170	16	66 (5 yr)	5.9 yr	Small lymphocytic histology
185	31	81 (3 yr)	Not reached	—
186	41	83 (4 yr)	56% still on watch and wait; median duration 24 months	Includes some intermediate histologies

combination chemotherapy, followed by 4,000 cGy of involved-field radiation.

Advanced Low-Grade Lymphomas

The treatment of advanced-stage low-grade lymphomas has been the subject of considerable debate, primarily centering around the question of curability of these patients and whether any therapy at all is indicated for asymptomatic patients.[168,181–183] Traditionally therapy for this group of patients has relied on three approaches: (1) watch and wait; (2) moderate therapy with limited chemotherapy or radiotherapy; and (3) aggressive chemotherapy and radiotherapy. The choice of therapy is more often based on the philosophy of the treating physician than on objective data.

Watch and Wait

In the light of studies showing a continuous relapse rate for aggressively treated patients with advanced low-grade lymphomas, many physicians choose to defer initial treatment in asymptomatic patients and adopt a conservative watch and wait approach. The long natural history of this disease and that patients are often elderly with coexisting medical problems make this approach attractive in many situations. There may be several potential benefits from withholding therapy initially in asymptomatic patients.[184] (1) It may be months or years after the initial diagnosis before therapy is required, and patients would almost certainly have a better quality of life without therapy during this time. (2) Withholding initial therapy will theoretically limit exposure to chemotherapeutic agents and, it is hoped, prevent resistance at a time when those drugs are truly needed. (3) Spontaneous regression of disease may occur, eliminating the need for treatment.[80] (4) Finally, it has been suggested that if those lymphomas transform into a more aggressive histology, treatment at that time might offer an improved chance of success.

The watch and wait approach has several potential disadvantages, however. Patients must be monitored closely to prevent insidious complications such as ureteral obstruction due to enlarging retroperitoneal adenopathy. It has also been suggested that waiting until the disease progresses and bulky adenopathy and systemic symptoms develop may make treatment at that time more difficult. Finally, many patients are unable to accept the option of letting their disease progress without therapy and insist on some form of treatment.

The natural history of patients managed with initial observation[80,81,170,185,186] is shown in Table 81-6. Overall survival in general is >5 years. Histologic transformation was seen in 6–15% of patients, while spontaneous remissions were seen in ≤23% of patients.[80] When patients were randomly assigned to the watch and wait approach versus aggressive chemotherapy and radiation, overall survival was not changed (83% versus 84%), while DFS was significantly influenced (0% versus 51%).[186] In another trial, which was nonrandomized, overall survival was significantly better in patients initially managed with a watch and wait approach.[81]

It is not certain whether aggressive initial therapy will influence overall survival. If patients are thought not to be candidates for such therapy and are asymptomatic and reliable, then an initial period of observation without treatment is acceptable. Factors associated with adverse prognosis in low-grade lymphoma patients have included advanced stage,[82,86,187] age,[82,187] and the presence of systemic symptoms.[61,81,86,170] The presence or absence of these factors may be helpful in deciding which patients can be initially managed with observation.

Moderate Therapy

Nonaggressive therapy has been used as initial therapy for some patients and as palliative therapy later in the course of disease in others. This approach has generally relied on single-agent alkylator therapy with chlorambucil or cyclophosphamide; combination chemotherapy with regimens such as CVP (cyclophosphamide, vincristine, prednisone); or radiotherapy. Single-agent alkylator therapy in patients with advanced disease has generally achieved response rates of 50–80%.[188–192] Cyclophosphamide may be given at a dose of 50–150 mg/day PO, or chlorambucil may be given at a dose of 0.1–0.2 mg/kg/day. Alternatively, chlorambucil may be given in pulses of 0.4–0.6 mg/kg every 2 weeks. Although this treatment is highly effective in controlling disease, patients are not cured and exhibit a continuous pattern of relapse.

CVP has been employed in several trials, and similar response rates of 80–90% have been observed.[190,193,194] Again, however, a continuous relapse pattern has been observed, and there is little evidence of cure in any of these patients. Trials comparing moderate with aggressive therapy have failed to show a survival advantage for patients treated aggressively.[77,168,182,186,190,194]

Radiation may also be extremely useful in managing patients with low-grade lymphomas,[195] who often develop localized problems related to painful adenopathy, cord compression, or ureteral obstruction. Those symptoms can be effectively palliated with local radiotherapy without resorting to systemic chemotherapy.

Aggressive Chemotherapy

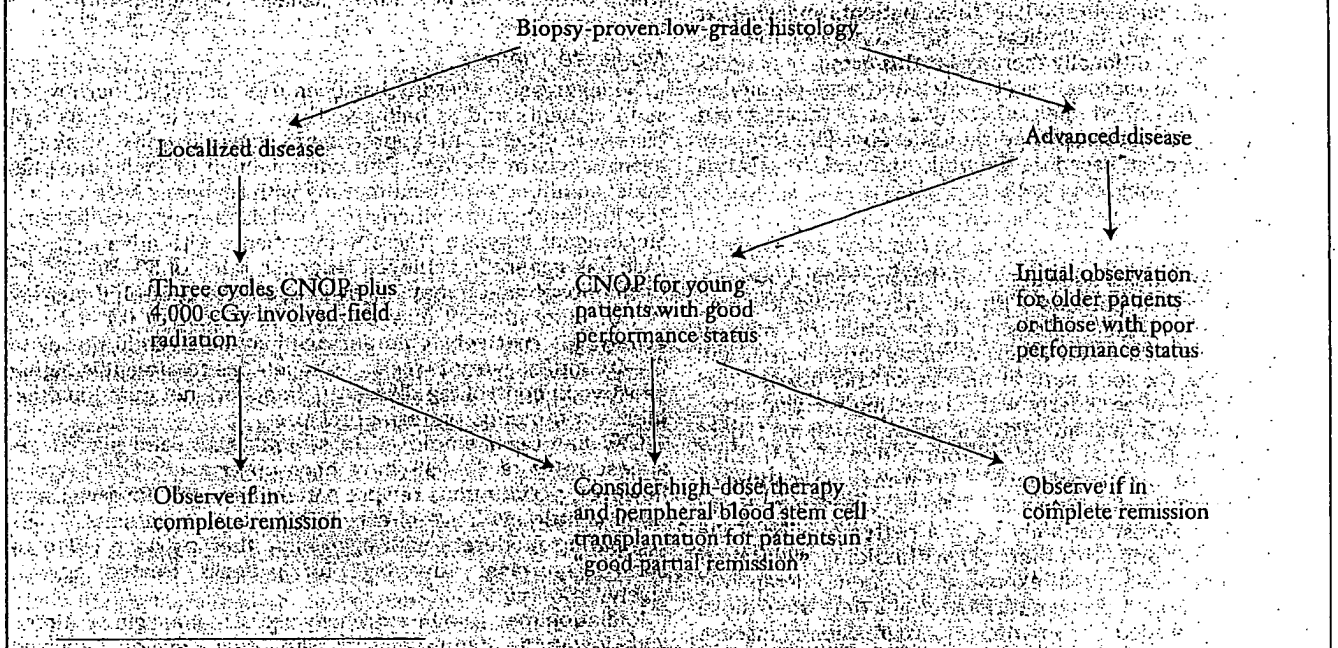
Despite the success of modern chemotherapy regimens for intermediate- and high-grade lymphomas, these regimens have been used relatively infrequently for low-grade histologies.[77,78,169,174,186,187,196–199] Table 81-7 reviews several studies in which low-grade lymphomas have been treated with somewhat more aggressive chemotherapy regimens. High complete remission rates have been observed in some series. Differences in reported remission rates may relate to various criteria for defining remission, and to differences in restaging intensity. Some studies appear to show a plateau in DFS, which may indicate cures, but late relapses are still seen, again bringing into question whether any of these patients are actually cured. Most studies show a continuous pattern of relapse, however. Although improved relapse-free survival is seen with aggressive therapy, it remains to be proven whether any difference in overall survival will be seen.

Two prospective randomized studies have shown higher rates of remission duration and overall survival when patients were treated with interferon in addition to intensive chemo-

NEBRASKA APPROACH TO THE INITIAL MANAGEMENT OF LOW-GRADE LYMPHOMA

Patients are first staged after diagnosis of a low-grade lymphoma. Our staging procedures include a history and physical examination, CBC, chemistry screen, chest radiograph, abdominal and pelvic CT scans, and a bone marrow biopsy. A chest CT is performed on patients with an abnormal chest radiograph. We do not use lymphangiography or perform a laparotomy on patients with clinically localized disease. If patients have localized disease (stage I or minimal stage II) they receive three cycles of CNOP[a] followed by 4,000 cGy involved-field radiation.

We explain the various treatment approaches to patients who present with advanced-stage disease. Younger patients (generally <60 years of age) with good performance status are generally offered aggressive therapy with CNOP with the goal of attaining a complete remission. Older patients, those with poor performance status or other medical problems, and patients who do not wish aggressive therapy are managed initially by a watch and wait approach. Palliative radiation or single-agent chemotherapy is used for symptomatic progression. Patients who achieve only a partial remission with initial anthracycline-based therapy are offered peripheral blood stem cell transplantation. Transplantation is also offered to patients who relapse.



[a] CNOP is identical to CHOP except mitoxantrone (12 mg/m² IV) is substituted for cyclophosphamide.

therapy.[198,199] Two purine analogues, fludarabine and 2-chlorodeoxyadenosine, have recently been introduced.[200–202] These new agents have shown high response rates in previously treated low-grade NHL. It is still unclear whether these drugs will become useful as initial therapy or in combination with other chemotherapy agents.

Aggressive Radiotherapy

Total body irradiation and total lymphoid irradiation have been used to treat patients with stage III and IV low-grade lymphomas.[203–205] Complete remission rates of 100% have been observed, along with 5-year DFS rates >60%. Although this approach is less often used than others, results are comparable with those of other forms of therapy.

Intermediate-Grade Lymphomas

For the purpose of this discussion intermediate-grade lymphomas include the follicular large cell, diffuse small cleaved, diffuse mixed, diffuse large cell, and immunoblastic histologic subtypes. The immunoblastic subtype is included in the intermediate category because of the lack of difference in response of diffuse large cell and immunoblastic lymphomas to modern, intensive chemotherapy regimens. Similarly, most investigators have not found a difference in outcome between diffuse mixed and diffuse large cell lymphoma. These three histologic subtypes are considered together.

Diffuse aggressive lymphomas are among the minority of neoplasms that can be cured by combination chemotherapy or, when localized, by radiotherapy. The treatment of these tumors has been one of the success stories of modern oncology. DeVita et al.[2] demonstrated that 37% of patients diagnosed as having diffuse histiocytic lymphoma could be cured with regimens previously demonstrated to be curative in patients with Hodgkin disease. A much smaller series of patients diagnosed with reticulum cell sarcoma had been reported 1 year earlier and seemed to demonstrate curability.[206] With the subsequent addition of doxorubicin and more recently etoposide, a large number of new regimens have been developed. The treatment of patients with localized and disseminated disease is considered in detail below.

Table 81-7. Aggressive Chemotherapy for Advanced Low-Grade Lymphomas

Reference	Patients (N)	Therapy	CR (%)	Follow-up	Comments
177	27	COPP	78	57% 5 yr PFS	Some received BCVP maintenance
	53	BCVP	64	26% 5 yr PFS	
178	74	CHOP-Bleo + IF	81	52% 5 yr RFS	Includes FLC, 78 mo median follow-up
174	64	C-MOPP	72	20/64 CCR	Median follow-up 7 yr
		CVP			
		TBI or TNI			
		MOPP			
		C-MOPP + TBI			
		BACOP			
		ProMACE-MOPP			
186	43	ProMACE-MOPP + TNI	78	25/43 CCR	4-yr median follow-up, includes intermediate histologies
	18	COPP	61	3/18 CCR	Median follow-up 3 yr
	14	BCVP	50	?	
197	18	M-BACOD	56	4/18 CCR	Median follow-up 58 mo, includes FLC
169	415	CHOP	64	6.9 yr median survival	Some received levamisole maintenance or chemotherapy maintenance
		CHOP + levamisole			
		CHOP + levamisole + BCG			
187	148	CHOP or CVP ± IF or TBI ± BCG	69		Some received chemotherapy maintenance
198	249	COPA ± Interferon	51		Improved outcome in interferon patients; includes some patients with intermediate histologies
199	242	CHVP ± Interferon	17		Improved outcome in interferon patients

Abbreviations: CR, complete remission; CCR, continuous complete response; PFS, progression-free survival; RFS, relapse-free survival; FLC, follicular large cell; COPP, cyclophosphamide, vincristine, procarbazine, prednisone; BCVP, BCNU, cyclophosphamide, vincristine, prednisone; M-BACOD, methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, prednisone; C-MOPP, cyclophosphamide, vincristine, procarbazine, prednisone; MOPP, nitrogen mustard, vincristine, procarbazine, prednisone; BACOP, bleomycin, doxorubicin, cyclophosphamide, vincristine, prednisone; ProMACE, prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide; CHOP/COPA, cyclophosphamide, doxorubicin, vincristine, prednisone; CHOP-Bleo, CHOP + bleomycin; CHVP, cyclophosphamide, doxorubicin, teniposide, prednisone; TBI, total body irradiation; TNI, total nodal irradiation; IF, involved field radiation.

Localized Diffuse Mixed, Diffuse Large Cell, and Immunoblastic Lymphoma

As might be expected, patients with localized minimal disease have a much better outlook than patients with more extensive disease. Patients with an especially good outlook and for whom less chemotherapy can be used are those with nonbulky (i.e., masses <10 cm) stage I or IE disease without systemic symptoms and occasional patients with stage II or IIE disease in whom the two sites of involvement are immediately adjacent and nonbulky and who are also without systemic symptoms. In such patients radiotherapy can be curative; however, the chances for cure with radiotherapy will be directly dependent on the thoroughness of the staging evaluation.[207] That is, patients who have undergone very aggressive staging procedures, including staging laparotomy, and are still found to have localized disease will have a significantly higher cure rate than patients who have undergone less aggressive staging. Because staging laparotomies are not often used in patients with aggressive NHLs, radiotherapy alone is not the preferred therapy for localized disease except in unusual situations, such as frail elderly patients, or those who refuse chemotherapy.

In patients treated initially with radiotherapy, a number of studies have demonstrated that following the radiotherapy with a course of chemotherapy reduces the frequency of relapse.[208–211] More recently, the opposite sequence of events has been studied (i.e., patients treated initially with an abbreviated course of chemotherapy followed by radiotherapy). When initial doxorubicin-based combination chemotherapy has been used, the long-term DFS rate has been very high.[212–215] Patients treated with an aggressive combination chemotherapy regimen for an abbreviated duration (e.g., three or four cycles rather than six) and then with involved-field radiotherapy have consistently showed a >80% DFS rate. A "full course" of chemotherapy alone is probably as effective as chemotherapy plus radiotherapy for patients with localized lymphomas.[213,216] This approach may be useful for patients who might have undesirable side effects from chemotherapy (e.g., dry mouth after radiation to the salivary glands).

Patients with certain sites of apparently localized, extranodal lymphoma should receive special attention. Those who present with lymphoma in the stomach or distal gastrointestinal tract are especially likely to have occult disease in Waldeyer's ring, and those who present with disease in Waldeyer's ring are likely to have disease more distally in the gastrointestinal tract. With either situation, a careful evaluation at the site of possible occult involvement is necessary before treating the patient for localized disease. Patients with testicular lymphoma should have the entire scrotal contents included in any radiation field to avoid the possibility of local relapse. Those with sinus or epidural lymphoma are at a high risk of central nervous system relapse and should be considered for prophylactic therapy to the central nervous system despite otherwise apparently localized disease. Patients who have radiotherapy to the thyroid are at high risk of eventually developing hypothyroidism and need to be followed expectantly.

Disseminated Disease

Several chemotherapy regimens can cure some patients with widely disseminated intermediate-grade NHL. However, certain principles must be followed in administering these drugs for them to be effective. The first principle is that the goal of therapy should be attainment of a complete remission as quickly as possible, as only patients who achieve a complete remission have any chance for cure with initial chemotherapy. The one possible exception to this rule is the patient who presents with bulky mediastinal or retroperitoneal disease with residual fi-

Table 81-8. Therapeutic Results for Diffuse Large Cell and Immunoblastic NHLs

Reference	Regimen[a]	CR Rate (%)	Relapse-Free Survival (%)	Overall Survival (%)
137	CAP-BOP	65	49	42 (3 yr)
148	LNH-84	75	70	67 (3 yr)
220	CHOP	53	60	30 (7 yr)
221	COP-BLAM	73	55	
222	MACOP-B	86	67	65 (6.5 yr)
223	m-BACOD	61	76	60 (5 yr)
224	ProMACE/Cyta-BOM	80	—	80 (2 yr)

[a] CHOP

	Days					
	1	5	10	15	22	
Cyclophosphamide 750 mg/m² IV	X					Repeat cycle
Adriamycin 50 mg/m² IV	X					
Vincristine 1.4 mg/m² IV	X					
Prednisone 100 mg PO	X	X	X	X		

COP-BLAM

	Days											
	1	5						10	14	21		
Cyclophosphamide 400 mg/m² IV	X											
Vincristine 1.4 mg/m² IV	X											
Prednisone 40 mg/m² PO × 10 days	X	X	X	X	X	X	X	X	X	X		Repeat cycle
Bleomycin 15 U IV											X	
Doxorubicin 40 mg/m² IV	X											
Procarbazine 100 mg/m² PO × 10 days	X	X	X	X	X	X	X	X	X	X		

MACOP-B

	Week of Therapy											
	1	2	3	4	5	6	7	8	9	10	11	12
Methotrexate 400 mg/m² IV		X				X				X		
Doxorubicin 50 mg/m² IV	X		X		X		X		X		X	
Cyclophosphamide 350 mg/m² IV	X		X		X		X		X		X	
Vincristine 1.4 mg/m² IV		X		X		X		X		X		X
Bleomycin 10 U/m² IV				X				X				X
Prednisone 75 mg PO	Daily dose tapered over the last 15 days											
Co-trimoxazole 2 tablets PO	Twice daily throughout											
Ketoconazole 200 mg PO	Once daily throughout											
Folinic acid 15 mg PO every 6 hr for 6 days, starting 24 hr after methotrexate												

m-BACOD

	Days									
	1	2	3	4	5	6	7	8	15	21
Methotrexate 200 mg/m² IV	X							X	X	Repeat cycle
Bleomycin 4 U/m² IV	X									
Doxorubicin 45 mg/m² IV	X									
Cyclophosphamide 600 mg/m² IV	X									
Vincristine 1 mg/m² IV	X	X	X	X	X					
Dexamethasone 6 mg/m² PO × 5 days										
Folinic acid 10 mg/m² PO every 6 hr for 8 doses, starting 24 hr after methotrexate										

(Table continues)

brotic masses.[135] Such patients can be identified by the existence of a mass that shrinks quickly early in therapy but thereafter remains stable in size. A gallium scan that goes from positive to negative can also be helpful in this determination.

Because salvage therapy is only marginally effective in these patients,[217] it is vital that patients be optimally managed initially and that a cure be achieved with the initial chemotherapy regimen if this is possible. This requires chemotherapy administered at full doses. Dose reduction for arbitrary reasons are never a favor to the patient. In studies in which reduced chemotherapy doses have been administered to patients with NHL, response rates have been significantly lower.[218]

It is not necessary to administer more than a few treatment cycles past the documentation of complete remission.[219] It is likely that prolonged therapy will increase treatment-related toxicity without increasing the chance for cure. New aggressive chemotherapy regimens reduce the treatment time to as few as 10–12 weeks in rapidly responding patients.

A summary of the results of several aggressive chemotherapy regimens with proven curative potential in aggressive NHL[137,148,220–224] is presented in Table 81-8. As can be seen, the complete remission rate varies from 53% to 86%, and the relapse-free survival for complete responders varies from 49% to 76%. Overall survival tends to be better in patients with shorter follow-up but averages approximately 60–75% at 3 years. The chances for cure can be obtained by multiplying the complete response rate by the relapse-free survival rate (i.e., the proportion of complete responders who do not relapse). This suggests that the cure rate for reported regimens might vary from approximately 30% with CHOP to approximately 60% with some newer regimens. However, it should be remembered that the CHOP regimen has the longest follow-up. In addition, it is difficult to compare the results of various trials due to differences in prognostic factors for patients in the trials. This has led to a prospective randomized trial comparing CHOP, m-BACOD, ProMACE-CytaBOM, and MACOP-B[225] (see Table 81-7 for definitions). No significant differences in response rates, time to treatment failure, or survival were noted

Table 81-8. *(Continued)*

ProMACE/Cyta-BOM	Days													
	1							8	9					14
Cyclophosphamide 650 mg/m² IV push	X													
Doxorubicin 25 mg/m² IV push	X													
Etoposide (VP-16) 120 mg/m² IV	X													
Prednisone 60 mg/m² PO × 14 days	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cytarabine 300 mg/m² IV push								X						Repeat cycle
Bleomycin 5 U/m² IV push								X						every 28 days
Vincristine 1.4 mg/m² IV push								X						
Methotrexate 120 mg/m² IV push								X						
Folinic acid 25 mg/m² PO every 6 hr × 6 doses									X					

LNH-84	Induction (wk)			Consolidation (wk)							Final Intensification (wk)		
	0	2	4	8	10	12	14	16	17	19	21	27	31
Cyclophosphamide 1,200 mg/m² IV	X	X	X									X	X
Doxorubicin 75 mg/m² IV	X	X	X										
Vindesine 2 mg/m² IV days 1,5	X	X	X										
Bleomycin 10 mg days 1,5	X	X	X									X	X
Prednisone 60 mg/m² IV × 5 days	X	X	X										X
Methotrexate 15 mg intrathecal	X	X	X										
Cytarabine 100 mg/m²/day SC × 4 days				X	X					X	X	X	X
Methotrexate 3 g/m² IV + leucovorin rescue 25 mg PO every 6 hr × 12 doses						X	X						
Etoposide 300 mg/m²						X	X						
Ifosfamide 1,500 mg/m²						X	X						
L-asparaginase 50,000 U/m² IM								X	X			X	X
Teniposide 60 mg/m² IV													

CAP-BOP	Schedule Days													
	1						7	15						21
Cyclophosphamide 650 mg/m² IV	X													
Doxorubicin 50 mg/m² IV	X													
Procarbazine 100 mg/m² PO × 7 days	X	X	X	X	X	X	X							Repeat cycle
Bleomycin 10 U/m² SC								X						
Vincristine 1.4 mg/m² IV								X						
Prednisone 100 mg PO × 7 days								X	X	X	X	X	X	X

among the various regimens. Several additional trials have failed to demonstrate the superiority of any particular chemotherapy regimen for NHL.[226] Many physicians are now using CHOP because of its ease of administration. The details of the administration of these, and other, regimens are presented in Table 81-8. Each regimen has proven curative potential in patients with diffuse large cell or other aggressive NHLs, and at present there is no basis for choosing one regimen over another.

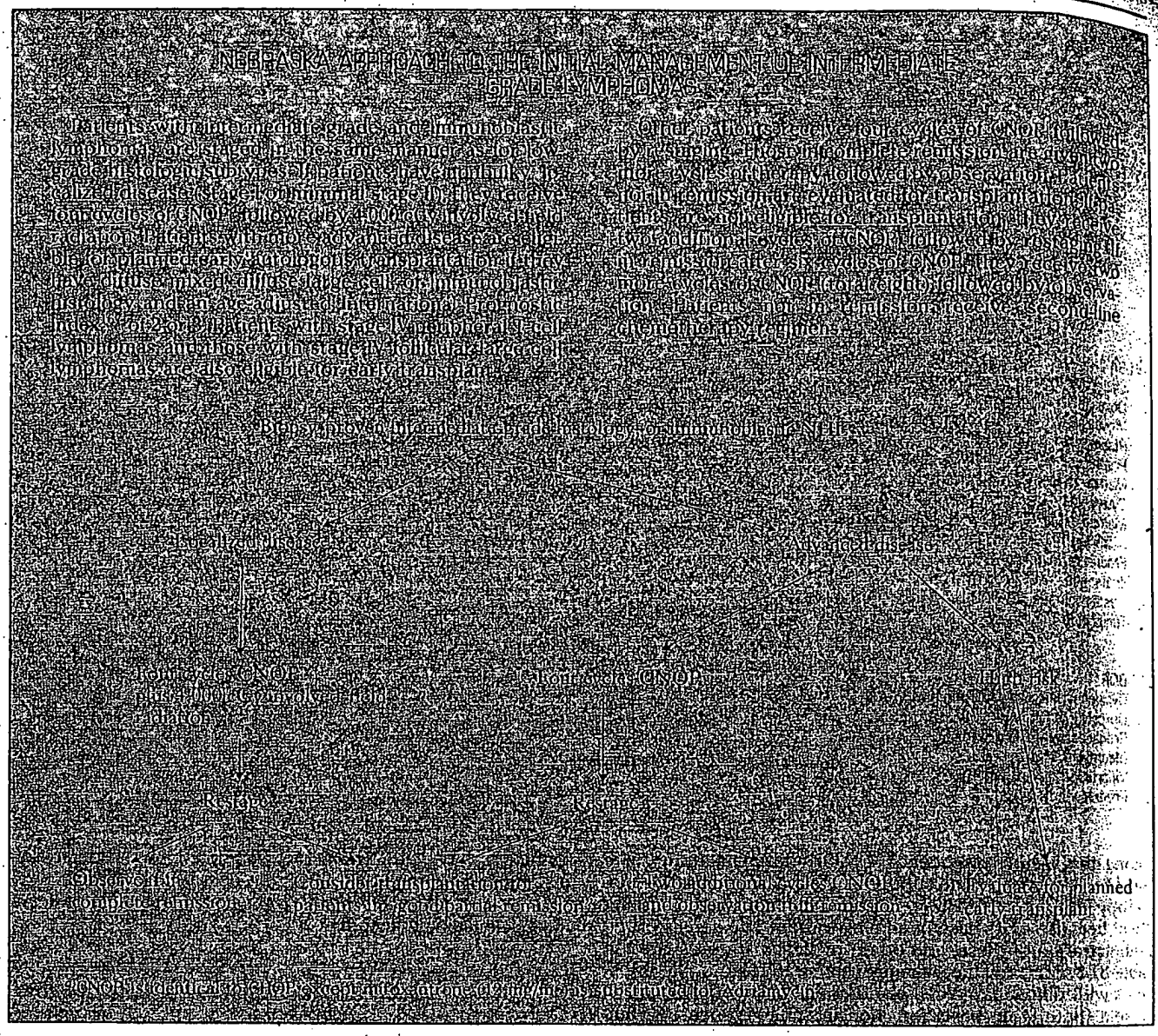
It is important to remember that the treatment of aggressive lymphomas with chemotherapy is fraught with risks. Almost all treatment trials have resulted in treatment-related deaths. Clinical factors that appear to predispose patients to increased risk of morbidity and mortality include older age and poor performance status. Familiarity with the regimen being used is important. Treatment-related mortality declines with increased experience in administering a particular chemotherapy regimen.[227] For this reason it is a logical plan for each oncologist to choose one regimen that has been proven to be active in aggressive NHLs and to use that regimen exclusively. It is hoped that advances in supportive care, such as hematopoietic growth factors, will lead to improved treatment results for NHL. Growth factors can allow increases in dose intensity for NHL patients and can decrease neutropenia and infection.[228,229] However, it is unknown whether this will result in improved remission rates or survival.

At our institution, once the patient has been carefully evaluated to determine the extent of disease, treatment is initiated. We use combination chemotherapy consisting of cyclophosphamide, mitoxantrone, vincristine, and prednisone (CNOP). This regimen may be less toxic than CHOP and has equivalent results.[230,231] Patients who achieve complete remission are followed closely in the first year but at decreasing intervals over the next several years. We do not routinely restage patients unless clinically indicated.

High-Grade Lymphomas

The treatment of lymphoblastic lymphoma with conventional chemotherapy regimens used for NHL has demonstrated excellent initial response rates in most trials. However, most patients relapse and eventually die of progressive disease that is unresponsive to salvage chemotherapy. In 1971 Aur et al.[232] reported encouraging results using an intensified chemotherapy program with induction maintenance and central nervous system prophylaxis for localized NHL in children, a heterogeneous group with a large proportion of cases of lymphoblastic disease. Additional studies in children evaluated the type of regimens used in ALL for treatment of lymphoblastic lymphoma. The LSA_2-L_2 protocol (used for ALL) was subsequently shown to be significantly more effective therapy than COMP (cyclophosphamide, vincristine, methotrexate, and prednisone) therapy for lymphoblastic lymphoma in children (76% 2-year failure-free survival for the LSA_2-L_2 protocol versus 26% for the COMP protocol).[233] Similarly, Weinstein et al.[110] reported a 69% 5-year actuarial survival for 21 patients, aged 2.5–22 years, with mediastinal lymphoblastic lymphoma treated with the APO (doxirubicin [Adriamycin], prednisone, vincristine) protocol.

Treatment of lymphoblastic lymphoma in adults is less well documented in the literature than the treatment of children



with this disease. Pediatric results have led to treatment principles in adults that use regimens similar to those used in ALL. Coleman et al.[234] reported a trial involving the treatment of 13 patients with mediastinal lymphoblastic lymphoma with an intensive chemotherapy protocol using central nervous system prophylaxis and achieved a complete response rate of 100%, with a 3-year actuarial DFS rate of 56%. In a study by Levine et al.,[108] 15 patients were treated with a modified LSA_2-L_2 protocol for adult lymphoblastic lymphoma. In this evaluation 73% of the patients achieved a complete response rate and the actuarial survival rate at 5 years for all patients was 40%. In a later trial by Coleman et al.,[235] it was possible to divide patients into good and poor prognostic groups based on the presence of marrow or central nervous system disease and a serum LDH level of >300 IU/ml. The good prognosis patients had a 5-year freedom from relapse rate of 94%, whereas the poor prognosis patients had a 5-year freedom from relapse rate of 19%. However, one other study, by Slater et al.,[236] failed to find any difference in survival based on bone marrow involvement. Results from a number of institutions (Table 81-9) have confirmed the poor results of treatment of lymphoblastic lymphoma in adults.

The poor results of treatment for adult lymphoblastic lymphoma have led to evaluation of the use of high-dose therapy with allogeneic or autologous BMT for hematopoietic reconstitution. One such trial from the City of Hope National Medical Center evaluated five patients with lymphoblastic lymphoma and one with diffuse undifferentiated lymphoma who were treated with high-dose cyclophosphamide and total body irradiation, with allogeneic BMT in first complete remission.[240] Four of the patients were alive in complete remission at 8, 14, 21, and 47 months post-transplant. One patient died of recurrent lymphoma 17 months post-transplant, and one died of graft-versus-host disease without evidence of lymphoma at autopsy. These encouraging results demonstrated that allogeneic BMT could produce durable remission in patients with high-grade lymphomas who present with bone marrow, central nervous system, and/or skin involvement. Subsequent studies have demonstrated that BMT in first complete remission may improve the outcome of adult lymphoblastic lymphoma patients.[241,242] It is unclear whether selection bias influenced these results, and randomized trials will be needed to determine the role of early BMT in lymphoblastic lymphoma.

As in lymphoblastic lymphoma, most therapeutic trials for

Table 81-9. Therapy Results for High-Grade NHL in Adults

Type	Reference	Regimen	CR Rate (%)	Disease-Free Survival (%)
Lymphoblastic	108	Induction: cyclophosphamide, doxorubicin, vincristine, prednisone, cytarabine, thioguanine, asparaginase, lomustine Maintenance: thioguanine, methotrexate, cyclophosphamide, hydroxyurea, doxorubicin, lomustine, cytarabine, vincristine	73	35 (5 yr)
	235	Induction: cyclophosphamide, doxorubicin, vincristine, prednisone, asparaginase Maintenance: methotrexate, mercaptopurine	95	56 (3 yr)
		Variable	78	45 (5 yr)
		Variable	74	30 (3 yr)
	236	Variable	53	—
	237	Variable	82	38 (2½ yr)
	238	Variable	77	71 (1 yr)
	239	Variable	80	60 (5 yr)
Small noncleaved cell	248	Cyclophosphamide, doxorubicin, vincristine, prednisone, methotrexate	85	60 (5 yr)
	249	Variable		
	250	Cyclophosphamide, etoposide, vincristine, bleomycin, doxorubicin, methotrexate, prednisone		

the treatment of SNCL have been carried out in the pediatric population, with few trials on adults available for analysis. In some initial African Burkitt lymphoma cases, long-term DFS with single-agent cyclophosphamide therapy has been reported. Several multiagent chemotherapy regimens combined with central nervous system prophylaxis have now been shown to achieve high remission rates and long-term DFS. Most of these protocols vary in their complexity and content but include cyclophosphamide combined with various other cytotoxic agents, including methotrexate, doxorubicin, cytarabine, and/or vincristine. The importance of central nervous system prophylaxis has now been documented in several trials, with decreased central nervous system relapse rates in the prophylactically treated patients. Protocols that were designed for lymphoblastic lymphoma or ALL (i.e., LSA_2-L_2 protocol) are clearly inferior to the specific SNCL protocols. This was confirmed in a Children's Cancer Study Group trial, in which children with SNCL had a much improved prognosis when treated with COMP as compared with those children treated with LSA_2-L_2.[233] Most trials report 50–75% overall survival rates in childhood SNCL with modern multiagent chemotherapy.[243–247] The differences in survival characteristics between trials can often be accounted for by differences in the patient populations. Trials containing older patients or more patients with central nervous system disease or bone marrow involvement would generate poorer survival characteristics. Patients with limited disease, such as resectable abdominal disease, have an excellent prognosis and may require a shorter treatment time or less intensive chemotherapy.

The therapy for SNCL in adults is less well defined. One study from Stanford reported 18 adult patients with SNCL who were treated with cyclophosphamide, doxorubicin, vincristine, prednisone, and systemic and intrathecal methotrexate. These investigators also used radiotherapy to treat unresectable masses of >10 cm when possible.[248] The patients with prognostic signs, including unresected tumor bulk >10 cm, pretreatment LDH >500 IU/L (normal ≤200 IU/L), or involvement of the central nervous system or bone marrow, had significantly worse results than patients without these features, their relapse-free survival rate being 28.6% versus 100%. Treatment results of small noncleaved-cell NHL in adults are shown in Table 81-9.

These results have pointed out that for children and adults who present with these poor prognostic signs, more intensive therapy is needed to expect a higher percentage of curability. The BACT (carmustine, cytarabine, cyclophosphamide, 6-thioguanine) massive chemotherapy regimen followed by autologous bone marrow rescue for the treatment of relapsed or resistant Burkitt lymphoma was first reported by Appelbaum et al.[251] in 1978. In the original report 14 patients were treated with this therapy, of whom three were alive at 9+, 19+, and 29+ months post-transplant. With improvement in the front-line treatment for Burkitt lymphoma, the indications for autologous BMT in Burkitt lymphoma have changed so that its use in poor prognosis, relapsed, or resistant patients is recommended at present. A 5-year experience in autologous BMT for Burkitt lymphoma was reported in 1986 by Philip et al.,[252] who performed BMT in 28 patients with Burkitt lymphoma using the BACT protocol or the BEAM (carmustine, etoposide, cytarabine, melphalan) protocol as high-dose chemotherapy. The overall DFS at the time of their report was 46%, with a median observation time post-transplant of 22 months. These excellent results were also achieved in the poor prognosis patients, with 5 of 10 long-term survivors having previous central nervous system disease. With conventional chemotherapy these results would have been difficult to achieve. Occult tumor cells are a potential problem when using autologous marrow for BMT in this setting. Explosive bone marrow relapse following autologous BMT for Burkitt lymphoma was reported in this trial as well as in other published trials. Also, it had previously been shown that Burkitt lymphoma cells can grow in a liquid culture system from cytologically and histologically normal marrow.[253] The role of marrow purging in this clinical setting is undergoing intensive evaluation. Alternatively, allogeneic marrow grafting may be useful in this clinical setting when a donor is available. Further clinical trials are ongoing to identify the patient population for whom high-dose therapy and BMT are appropriate.

The high-grade NHLs have distinct biologic properties that make timely evaluation and treatment important. Modern chemotherapeutic regimens and improvement with salvage treatments such as high-dose chemotherapy and autologous or allogeneic BMT have greatly improved the outlook for patients with these lymphomas. It is hoped that new trials will improve the survival of this difficult patient population.

Salvage Therapy

Despite the progress observed with first-line chemotherapy regimens for NHL, ≥30–50% of patients will not achieve remission. These patients have a poor prognosis, and almost all will die of progressive lymphoma without effective second-line (salvage) therapy.

Conventional Chemotherapy

Table 81-10 shows the results from several recent salvage chemotherapy regimens for NHL.[254–259] Although relatively high complete response rates are noted, <10% of patients with relapsed or refractory NHL will achieve long-term DFS. Numerous other regimens have been employed for relapsed or refrac-

NEBRASKA APPROACH TO THE INITIAL MANAGEMENT OF HIGH-GRADE LYMPHOMAS

Lymphoblastic NHL is treated with the following Regimen A:

Agent	Pre	Induction	Prophylaxis	Consolidation	Maintenance
	Week 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22–52				
Cyclophosphamide 1.0 gm/m² IV	X	X	X		
Doxorubicin 50 mg/m² IV	X	X	X		
Vincristine 2.0 mg IV	X X X X X X X		X	Repeat weeks 9–12 × 3	
Prednisone 40 mg/m² PO	←daily→	taper	5 days		
L-asparaginase 6,000 U/m² IM or IV (max 10,000 U)	6 doses				
Methotrexate 12 mg IT	X	X	←5 doses→		
Whole brain XRT 2,400 cGy in 12 fractions		XRT→			
Methotrexate 30 mg/m² PO					Weekly
Mercaptopurine 75 mg/m² PO					Daily

[1]Patients are evaluated for autologous or allogeneic BMT at the end of consolidation.

[2]Patients with small noncleaved histology are treated like advanced-stage intermediate histology patients. Those with bulky disease, bone marrow involvement, central nervous system involvement, or high LDH are evaluated for autologous or allogeneic BMT in first complete remission or at best response.

tory lymphomas using both available and investigational agents.[217] Other treatment strategies such as infusional chemotherapy,[260,261] and chronic administration of oral etoposide have also been tried.[262] Similar response rates are seen, but only rare patients appear to be cured of their disease. The poor results are due to primary drug resistance following failure of front-line therapy and to the inability of patients to tolerate full doses of chemotherapy salvage regimens.

Marrow Transplantation

For the reasons cited above, high-dose therapy with BMT is now being used with increasing frequency to treat patients with relapsed or refractory NHL.[263] This technique allows delivery of radiation or chemotherapy in higher than normal doses, thereby taking advantage of the steep dose-response curves exhibited by these agents against lymphomas.[264–266] This dose escalation makes it possible to overcome drug resistance without regard for what might otherwise be lethal bone marrow toxicity, since patients can subsequently be rescued with syngeneic, allogeneic, or autologous marrow.

Syngeneic and allogeneic transplants for NHL have been performed relatively infrequently compared with autologous transplants.[267–274] It is clear, however, that a substantial proportion of patients can be cured with this technique and that results are superior when patients undergo BMT in a state of minimal disease. No significant differences in overall survival

Table 81-10. Salvage Chemotherapy Regimens for NHL

Reference	Drugs	Patients (N)	CR (%)	PR (%)	Follow-up
254	Etoposide Methylprednisolone Cytarabine Cisplatin	24	(CR + PR = 69%)		—
255	Dexamethasone Cytarabine Cisplatin	90	28 (31)	22 (24)	20, CCR median follow-up 11 mo
256	Ifosfamide Methotrexate Etoposide	52	19 (37)	13 (25)	10, CCR 12 mo median projected RFS for CR patients
257,258	Methyl-GAG Ifosfamide Methotrexate Etoposide	208	49 (24)	75 (36)	Approximately 25% of CR patients in CCR
259	Dexamethasone Ifosfamide Cisplatin Etoposide	22	6 (22)	11 (50)	—

Abbreviations: CR, complete response; CCR, continuous complete response; PR, partial response; RFS, relapse-free survival; TTR, time to relapse.

Table 81-11. Autologous BMT in NHL

Reference	Patients (N)	Histologies	Therapy	CR (%)	CCR
276	100	Intermediate and high grade	Variable	57 (57)	19 (21–75 mo)
277	50	Intermediate and high grade	Variable	15 (30)	6 (19–45 mo)
278	46	Low, intermediate, and high grade	Variable	—	25 (8–104 mo)
279	68	Intermediate and high grade	Cyclophosphamide and TBI	37 (54)	15 (37–109 mo)
280	70	Low, intermediate, and high grade	Variable	51 (73)	16 (12–78 mo)
281	44	Low, intermediate, and high grade	Cyclophosphamide, etoposide, and TBI	—	25 (14–84 mo)

Abbreviations: CR, complete remission; CCR, continuous complete remission; TBI, total body irradiation.

have been observed between syngeneic, allogeneic, and autologous BMT for NHL,[272–274] except that allogeneic BMT results may be better for patients with lymphoblastic lymphoma.[274] Allogeneic BMT eliminates the risk of infusing malignant cells back with the marrow. In addition, allogeneic BMT has the advantage of a possible graft-versus-lymphoma effect similar to the graft-versus-leukemia effect that has been observed after allogeneic BMT for leukemia.[275] Studies do show a lower relapse rate for allogeneic marrow recipients, compared with autologous marrow recipients.[273,274] This suggests that a graft-versus-lymphoma effect exists; however, the effect is offset by higher transplant-related mortality with allogeneic BMT. Autologous BMT eliminates the need to find a matched donor, can be more safely performed in older patients, and eliminates the problems of graft-versus-host disease found in allogeneic BMT. Although there is a theoretical risk of infusing malignant cells with autologous BMT, this technique, rather than allogeneic BMT, is the one most often used.

Table 81-11 shows the results of several large studies of autologous BMT for NHL.[276–281] Approximately 20–40% of patients experience prolonged DFS with this procedure and appear cured. Although mortality rates have averaged 15–20%, the safety of BMT has improved due to advances in supportive care such as hematopoietic growth factors. Retrospective evidence suggests that results of BMT are better than those of conventional salvage chemotherapy,[282] and prospective trials are under way to test this hypothesis.[283] Patients who receive transplants after relapse but whose tumors still respond to conventional chemotherapy (sensitive relapse) have superior results compared with those whose tumors do not respond to conventional chemotherapy (resistant relapse).[276–281] Current studies are attempting to define patients who might benefit from earlier BMT. Patients who fail to enter complete remission, or who respond slowly to initial chemotherapy, have a very poor prognosis. Several studies have shown that these patients may have an improved outcome if they are transplanted in first partial remission.[277,278,281] In addition recent evidence suggests that patients with poor prognostic features may have improved survival if transplanted in first complete remission.[284,285] There is now increasing use of autologous peripheral stem cells instead of autologous bone marrow for hematopoietic recovery after high-dose therapy. This technique allows patients to undergo BMT if their marrow contains malignant cells or if their marrow cannot be harvested (e.g., after radiation to the pelvis). Autologous peripheral stem cell transplantation has been used for relapsed NHL, and results are at least as good as those of BMT.[286] Most autologous transplants for NHL have been performed in patients with intermediate- and high-grade histologies. Comparatively few transplants have been performed for low-grade lymphomas. The results of these transplants show that complete remissions can be obtained, but these patients will need prolonged follow-up to determine whether this modality can effect a cure.[287–289]

Other Approaches

Several studies have examined the use of recombinant interferon for NHL.[290–292] Responses have been seen at various dosages and seem to occur in approximately 50% of patients with low-grade NHL and those with cutaneous T-cell lymphoma, even among heavily pretreated patients. Few responses have been noted with intermediate- and high-grade lymphomas. Recent evidence suggests that results of therapy for NHL may be improved by adding interferon to conventional chemotherapy regimens.[198,199]

Finally, the use of monoclonal antibodies is being investigated in NHL. Such antibodies might be used in various ways. Anti-idiotype antibodies can be developed that are directed against the unique variable region of the immunoglobulin molecule on the lymphoma cell surface. Such antibodies are primarily directly cytotoxic or impair the tumor cell proliferation. Responses were seen in 9 of 15 patients treated with this approach in one study.[293] In addition, it may be possible to use immunoglobulin idiotype as an antigen to form lymphoma vaccines.[294] Monoclonal antibodies might also be used to deliver toxins, radioactive isotopes, or chemotherapeutic agents specifically to tumor cells. Anti-B-cell antibodies labeled with ^{131}I have shown activity in refractory NHL.[295–297] Antibodies conjugated to various plant toxins are being investigated for activity in patients with relapsed NHL.[298–299]

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